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A longitudinal study on the effect of obesity upon circulating renin-angiotensin system in normal pregnancy

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Abstract *Background and aims:* Obesity is the most common health issue in women of reproductive age, which profoundly affects maternal-fetal health. Despite progress in understanding key inflammatory and metabolic changes, the pathogenesis of the cardiovascular phenotype of obese pregnant women remains to be fully understood. This study aimed at: (*i*) evaluating the changes of the renin-angiotensin system (RAS) throughout pregnancy in obese vs normal weight (control) women, and (*ii*) evaluating the presence of any associations between maternal hemodynamic status and RAS changes.

Methods and results: Thirty-eight normal weight and nineteen obese pregnant women were included. Clinical assessment, blood samples and maternal hemodynamic evaluation were performed at 12, 20, 30, and 36 weeks, while ultrasound assessment was scheduled at 20, 30, and 36 weeks of gestation. Measurements of sFlt-1, PlGF, Angiotensinogen, Renin, AngII, Ang1-7, ACE and ACE2 were performed by ELISA. Our data show that normotensive obese women had lower placental blood supply, as assessed by UV-Q and UV-Q/EFW, as compared to controls, and significantly higher levels of AngII and AngII/Ang1-7 ratio, which were inversely related to placental blood supply.

Conclusions: Our study shows for the first time that normotensive obese women exhibited a significant progressive increase of AngII and AngII/Ang1-7 throughout pregnancy, which were inversely related to placental blood supply as assessed by UV-Q and UV-Q/EFW. Our data shed light on the early changes in pregnant obese women and suggest that RAS dysregulation is a prerequisite rather than a consequence of hypertensive disorders of pregnancy and other maternal neonatal complications.

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1. Introduction

Obesity is the most common health issue in women of reproductive age, which profoundly affects maternal-fetal health [1]. Obesity in pregnancy, defined as a body mass index (BMI) of $\geq$30 kg/m^2, leads to an increased risk for various maternal and neonatal complications, such as hypertensive disorders of pregnancy, gestational diabetes mellitus, cesarean delivery, pre-term birth, fetal macrosomia, intrauterine fetal demise, congenital abnormalities [2], and to a risk of both small and large for gestational age babies [3]. Despite progress in understanding the key inflammatory and metabolic changes, the pathogenesis of the cardiovascular phenotype of obese pregnant women remains to be fully understood.

The activation of the renin-angiotensin system (RAS) is considered one of the mechanisms underlying the development of hypertensive disorders of pregnancy [4] (although it remains to be established if RAS dysregulation is a prerequisite or a consequence of the development of hypertensive disorders of pregnancy) [5]. The RAS is a complex network of enzymes and peptides, whose main effector is Angiotensin II (AngII) that binds to its specific receptors AT1R and AT2R, whereby it regulates cardiovascular function and body fluid homeostasis, through vasoconstriction, liquid reabsorption, and aldosterone secretion. Locally, AngII regulates cell growth, inflammation, and fibrosis. AngII effects are offset by angiotensin-converting enzyme 2 (ACE2), which is an enzyme that cleaves AngII to generate Ang1-7, a vasodilating peptide with peripheral opposite actions to those of AngII [6]. In pregnancy, the feto-placental unit is an important additional site of RAS activity [7], and a healthy pregnancy outcome depends on the balanced activation of the RAS at a circulating and tissue level [4]. For instance, mice with deletion of ACE2 display 3-fold higher AngII content in the placenta, high blood pressure, reduced weight gain during pregnancy, fetal growth restriction [8].

Obese women seem to have a dysregulation of the RAS because various elements of this system have been identified in adipose tissue, which is a significant source of angiotensinogen and AngII [9,10]. Maternal overweight reduces the expression of neprilysin in the feto-placental endothelium, which may alter the balance of vasoactive peptides [11]. In addition, overweight women with pre-eclampsia exhibit a reduction of plasma ACE2 as well as ACE2 activity, in line with the view that unbalanced changes of ACE and ACE2 throughout pregnancy could be involved in the pathogenesis of maternal hemodynamic complications [12].

Based on these premises, we hypothesized that RAS is dysregulated in obese pregnant women and this study aimed at (*i*) evaluating the changes of the RAS throughout pregnancy in normal weight (control) and obese women (case), and (*ii*) to evaluate the presence of any associations between maternal hemodynamic parameters and RAS changes.

2. Methods

2.1. Study design

This is a prospective observational case-control study, whose primary outcomes were: (i) to describe the changes of the RAS throughout normal pregnancy in normal weight (control) and obese women (case); (ii) to evaluate the presence of any associations between maternal hemodynamic parameters and RAS changes. This study was conducted in accordance with the declaration of Helsinki, and it was approved by the Institutional Review Board and the local Ethics Committee [CEUR-2019-SPER-113].

Between March 2019 and November 2021, pregnant women with singleton pregnancy were recruited consecutively from the first trimester screening test for major aneuploidies (between gestational week 11–13) in a single tertiary referral center. Being a prospective study on pregnant women, patient follow-up ended in September 2022. Inclusion criteria of patients were: (i) age >18 years; (ii) spontaneous pregnancy; (iii) first trimester dating based on crown-rump length measurement; (iv) patient consent to participate in the study; as well as (v) BMI $\leq$24.9 for normal weight women (controls) and BMI $\geq$30 for obese women (cases). Exclusion criteria were: (i) multiple pregnancy; (ii) heterologous pregnancy; (iii) high-risk pregnancy and/or presence of other comorbidities (e.g. diabetes); (iv) smoking history. Although the protocol was written before COVID-19 outbreak, after February 2020 we excluded also women with history of COVID-19 (including history of positive PCR test for SARS-CoV2 from nasal swab). After informed consent, all pregnant women were scheduled to undergo clinical and laboratory assessment, as well as the measurement of hemodynamic parameters with the non-invasive Ultrasonic Cardiac Output Monitor (USCOM) at 12, 20, 30, and 36 weeks ($\pm$1 week) of gestational age. Ultrasound assessment of fetal biometry and Doppler velocimetry was performed at 20, 30 and 36 weeks ($\pm$1 week); moreover, for the purpose of this study, umbilical vein and uterine artery blood flow volume were computed.

2.2. Clinical assessment

The clinical assessment aimed at evaluating body weight, body weight gain, body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP), which were measured with an aneroid sphygmomanometer. In addition, we collected information regarding medication and any maternal complication, such as hypertensive disorders of pregnancy and gestational diabetes mellitus (GDM), miscarriage, gestational age at delivery, and premature birth. Screening for GDM was based on the 75 g oral glucose tolerance test and evaluated according to International Federation Gynecology and Obstetrics guidelines. After delivery, birth weight was measured and we collected information regarding birth mode, Apgar at 1

and 5 min, need of intensive care unit support, congenital malformations, infant sudden death, small for gestational age (SGA) and large for gestational age (LGA).

2.3. Laboratory assessment

Blood sampling was performed at 08.00 a.m., after an overnight fasting, to measure: soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF), angiotensinogen, renin, ACE, ACE2, AngII, and Ang1-7. It has to be noted that the angiogenic factors sFlt-1 and PlGF do not belong to the RAS and they were measured based on current guidelines because they are considered maternal biomarkers that help predict or diagnose placenta-related disorders, including pre-eclampsia, fetal growth restriction, stillbirth and preterm birth [13]. All measurements were performed by ELISA (sFlt-1: R&D Systems, Cat # DY321B; PlFG: R&D Systems, Cat # DY264; Angiotensinogen: LSBio, Cat # LS-F13066; renin: R&D Systems, Cat # DY4090; ACE: Elabscience, Cat # E-EL-H6001; ACE2: Elabscience, Cat # E-EL-H0281; AngII: Elabscience, Cat # E-EL-H0326; Ang1-7: Elabscience, Cat # E-EL-H5518). The intra-assay coefficients of variations were <5.1 % for sFlt-1, <5.5 % for PlGF, <4.7 % for angiotensinogen, <6.0 % for renin, <5.4 % for ACE, <6.0 % for ACE2, <6.6 % for AngII and <6 % for Ang1-7. The inter-assay coefficients of variations were <7.5 % for sFlt-1, <8 % for PlGF, <7.5 % for angiotensinogen, <7.4 % for renin, <5.4 % for ACE, <5.4 % for ACE2, <6.1 % for AngII and <5.2 % for Ang1-7.

2.4. Hemodynamic assessment

Non-invasive assessment of maternal hemodynamic status was performed with the USCOM device (USCOM Ltd. Coffs Harbour, Australia). USCOM employs continuous-wave Doppler, with the Doppler transducer placed at the suprasternal notch and the ultrasound beam directed in 3 planes to maximise the velocity of the aortic valve blood flow. Using an anthropometric algorithm, which correlates the outflow tract diameter with the patient's height, USCOM uses the velocity-time integral to compute hemodynamic parameters. In this study, we measured stroke volume (SV), cardiac output (CO), and systemic vascular resistance (SVR). USCOM measurements were performed under standardized conditions for the entire cohort, as already described [14], on patients lying in a semi-recumbent position and after a period of rest. All women had an USCOM assessment four times during gestation. All measurements were performed by the same trained researcher (M.B.).

2.5. Ultrasound assessment

All examinations were performed transabdominally, using Voluson E8 or Voluson E10 ultrasound systems (GE Healthcare, Zipf, Austria) with a convex abdominal array 2.5–5 MHz MFI (multifrequency imaging) probe. After the enrollment between week 11–13, all women had 3 ultrasound assessments at 20, 30, and 36 (±1 week) of gestation. As per local protocol, pregnant women underwent a complete assessment of maternal and fetal wellbeing. Ultrasound examination included: fetal biometry (head circumference [HC], biparietal diameter [BPD], abdominal circumference [AC], femur length [LF], and estimated fetal weight [EFW]), and Doppler velocimetry (uterine arteries [UtA], umbilical artery [UA] and middle cerebral artery [MCA]). Fetal biometry and feto-placental Doppler velocimetry were performed following the ISUOG guidelines [15,16]. The estimated fetal weight (EFW) was calculated by using the Hadlock formula [17]. For the purpose of this study, uterine arteries (UtA) and umbilical vein (UV) blood flow volume (Q) both absolute and normalized for estimated fetal weight (UtA-Q/EFW; UV-Q/EFW) were calculated as reported previously [18].

2.6. Statistical analysis

Sample size was calculated with openepi.com. To detect a mean difference in AngII of 350 pg/mL with a standard deviation of 375 pg/mL [19], and a two-sided significance level of 5 % with a power of 80 % would require 19 patients in each group. Based on this estimate, we decided to double the number of controls to obtain a ratio 2:1.

All statistical analyses were carried out in R system for statistical computing (Version 4.0.2; R development Core Team, 2020). Statistical significance was set at $p < 0.05$. Shapiro-Wilk test was applied to continuous variables to check for distribution normality. Quantitative variables were reported as median with range (min-max) or mean ± standard deviation, depending on distribution. Categorical variables were reported as absolute frequencies and/or percentages. Quantitative variables were compared by t-test or Mann-Whitney test, depending on data distribution, whereas categorical variables were compared by Chi-Square test at different time-points. To detect statistically significant differences between the different time intervals (time effect) and simultaneously compare the two groups (group effect), we used ANOVA-type statistics (ATS), which is a paired nonparametric longitudinal data analysis (R packages: nparLD). The analysis was based on the values of the F test statistic. The resulting F was compared to the F critical value obtained as a function of numerator and denominator degrees of freedom and $\alpha = 0.05$. In particular, denominator degrees of freedom were set to infinity by default. F values below the critical value identified non-significant results. For the overall group-effect the critical value was 3.8. For the effect of time alone and for group–time interaction, critical values varied between 2.6 and 3.8 depending on the considered variable.

Linear associations were evaluated with the Pearson or Spearman coefficient and subsequent multivariate linear regression models.

3. Results

3.1. Women characteristics

A total of 38 normal weight women (controls) and 19 obese women (cases) were recruited. Demographic, obstetric, and neonatal characteristics of all women are shown in Table 1. BMI was 22 in control women, while it was 33 in obese women. Throughout pregnancy obese women put on less weight as compared to control women (5.9 kg vs 10.9), in line with the optimal ranges of gestational weight gain for BMI categories that recommend an increase of 5–9 kg for obese women [20]. All women were normotensive, but obese women had a higher percentage of gestational diabetes (63.2 % vs 10.5 %, $p < 0.001$). Gestational diabetes was managed with insulin in 33 % obese women (4/12), while in the remaining 67 % obese (8/12) and in all control women (4/4) it was managed with lifestyle (diet and physical activity). In general, all pregnant women were encouraged to get 30 min of physical activity every day. All women had normal singleton pregnancy outcome and gave birth between 37 and 42 week of gestation. There were no significant differences in birth weight or other neonatal outcomes between the two groups.

3.2. Hemodynamic parameters

Cross-sectional comparisons between pregnant obese and control women at each time point are shown in Table 2 and Fig. 1. Results of longitudinal paired comparisons within and between the groups are shown in Table 3. Overall, all women were normotensive, as assessed by office blood pressure measurement. Likewise, SV, CO, and SVR did not differ between obese and control women throughout pregnancy. Maternal ultrasound assessment showed that mean uterine artery blood flow (UtA-Q) significantly increased (time effect F 13.8, $p < 0.001$), while mean uterine artery pulsatility index (UtA-PI) significantly decreased (time effect F 50.7, $p < 0.001$) throughout pregnancy, with no changes between the groups (Fig. 1A and Table 3). Also umbilical vein blood flow (UV-Q) significantly increased over time (time effect F 331.8, $p < 0.001$), with a group difference. In particular, at 30 weeks of gestation, obese women had significantly lower UV-Q as compared to controls (160 mL/min vs 205 mL/min, $p = 0.04$), and they had a significantly lower UV-Q increase from week 20–30 as compared to controls (group effect F 5.4, $p = 0.03$) (Fig. 1B and Table 3). On the other hand, umbilical vein blood flow adjusted for estimated fetal weight (UV-Q/EFW) significantly decreased

Table 1 General characteristics.

	Normal weight n = 38	Obese n = 19	p-value
Maternal Age (years)	31.5 (20; 41)	32 (25; 42)	0.68
Maternal weight (Kg)	60.7 (47.5; 74.5)	92.9 (73.3; 122.5)	<0.001
Maternal BMI	22 (17.5; 24.2)	32.6 (30.9; 40.5)	<0.001
Gestational age at first scan (wks)	12.4 (11.4; 13.6)	12.1 (11.6; 12.9)	0.10
Gestational weight gain (kg)	10.9 (6.5; 15.9)	5.9 (1.5; 10.4)	<0.001
From 12 to 20 weeks	3.1 (−0.5; 12.3)	1.3 (−2; 4)	<0.001
From 20 to 30 weeks	4.4 (−6; 9)	3.5 (−0.9; 8)	0.068
From 30 to 36 weeks	3 (0; 8)	1.5 (0; 3)	<0.001
Fetal sex			0.121
Male	23 (60.5 %)	6 (37.5 %)*	
Female	15 (39.5 %)	10 (60.5 %)*	
Pre-existing diabetes			
Yes	0 (0 %)	0 (0 %)	
No	38 (100 %)	19 (100 %)	
Pre-existing Hypertension			
Yes	0 (0 %)	0 (0 %)	
No	38 (100 %)	19 (100 %)	
SBP (mmHg)			
At 12 weeks	113 (90; 130)	120 (100; 130)	0.148
At 20 weeks	119 (98; 135)	110 (80; 138)	0.137
At 30 weeks	111 (89; 138)	110 (100; 130)	0.993
At 36 weeks	116 (87; 134)	110 (100; 130)	0.313
Gestational Hypertention			
Yes	0 (0 %)	0 (0 %)	
No	38 (100 %)	19 (100 %)	
Gestational diabetes			<0.001
Yes	4 (10.5 %)	12 (63.2 %)	
No	34 (89.5 %)	7 (36.8 %)	
Gestational age at delivery (wks)	40 (37–42)	40 (37–41)	0.808
Cesarean delivery	3 (9 %)	3 (19 %)	0.237
Newborn birth weight (g)	3350 (2670; 4310)	3465 (2320; 4090)	0.754
Small for gestational age	2 (10.5 %)	4 (12.5 %)	0.833
Large for gestational age	3 (10 %)	3 (19 %)	0.401
Apgar 5	10 (8–10)	10 (9–10)	0.924

BMI is for body mass index; SBP is for systolic blood pressure.

Table 2 Cross-sectional comparisons between obese and control women throughout pregnancy.

Variables	12 weeks			20 weeks			30 weeks			36 weeks		
	Normal weight N = 38	Obese N = 19	p-value	Normal weight N = 38	Obese N = 19	p-value	Normal weight N = 38	Obese N = 19	p-value	Normal weight N = 38	Obesity N = 19	p-value
SBP	112 (105–116)	110 (110–120)	***0.471***	114 (106–122)	110 (100–110)	***0.261***	108 (104–114)	100 (100–120)	***0.595***	117 (112–124)	110 (105–110)	***0.012***
DBP	66 (65–71)	67 (65–70)	***0.637***	65 (60–70)	70 (60–75)	***0.331***	67 (58–70)	75 (70–75)	***0.056***	68 (63–73)	70 (70–75)	***0.272***
SV	71 (60–79)	72.5 (63–86)	***0.544***	83 (68–94)	72.5 (60–87)	***0.437***	72 (69–82)	66 (55–80)	***0.466***	75 (64–80)	65.5 (54–77)	***0.308***
CO	4.9 (4.0–5.7)	5.9 (5.0–6.9)	***0.215***	5.5 (5.1–5.8)	6.0 (5.0–6.7)	***0.465***	5.3 (4.7–5.9)	6.1 (5.0–7.6)	***0.244***	5.7 (5.4–6.0)	6.0 (4.7–6.5)	***0.981***
SVR	1383 (1077–1501)	1170 (1088–1515)	***0.804***	1139 (1071–1256)	1257 (926–1466)	***0.852***	1434 (1086–1487)	1073 (925–1259)	***0.072***	1224 (1069–1243)	1159 (948–1291)	***0.420***
UtA-Q mean	NA	NA	**NA**	104.9 (74.9–148.8)	89.3 (65.2–138.6)	***0.743***	179.0 (104.5–231.3)	147.0 (59.9–231.8)	***0.213***	187.9 (109.8–258.8)	140.2 (96.1–178.1)	***0.233***
UtA-Q mean/EFW	NA	NA	**NA**	288.4 (229.4–404.8)	257.1 (195.9–415.1)	***0.796***	113.9 (77.7–138.1)	99.4 (37.1–143.3)	***0.228***	70.5 (44.1–102.2)	54.9 (33.6–71.3)	***0.208***
UtA-PI	NA	NA	**NA**	0.97 (0.82–1.11)	0.83 (0.72–0.87)	***0.170***	0.69 (0.58–0.73)	0.69 (0.54–0.85)	***0.852***	0.64 (0.58–0.83)	0.65 (0.51–0.75)	***0.882***
UV-Q	NA	NA	**NA**	49.0 (41.2–64.0)	40.7 (34.9–47.5)	***0.065***	205.2 (163.0–265.8)	159.9 (149.1–191.2)	***0.039***	256.9 (215.4–342.2)	239.6 (213.6–289.3)	***0.405***
UV-Q/EFW	NA	NA	**NA**	138.0 (123.3–164.8)	115.4 (101.1–136.9)	***0.087***	131.5 (111.8–158.1)	103.7 (88.5–115.0)	***0.023***	101.0 (84.7–115.4)	92.5 (77.4–111.9)	***0.335***
sFlt-1	2100.8 (1111.2–4100.3)	150.9 (97.5–634.3)	***<0.001***	2003.5 (1005.5–3493.2)	997.4 (198.9–2829.1)	***0.041***	2586.2 (1757.8–3433.8)	3641.7 (2776.8–4268.9)	***0.045***	5071.5 (2922.0–6804.1)	3139.9 (1996.5–5304.9)	***0.707***
PlGF	81.8 (40.9–290.8)	19.2 (6.4–38.5)	***0.006***	263.5 (143.7–459.3)	213.2 (101.7–1240.5)	***0.618***	603.9 (368.3–836.9)	331.0 (260.7–1835	***0.751***	408.5 (267.4–638.9)	462.1 (183.4–877.5)	***0.892***
sFlt-1/PlGF	25.6 (9.8–86.2)	7.9 (2.5–93.1)	***0.618***	12.4 (5.8–23.1)	12.7 (4.8–26.2)	***0.892***	9.5 (3.5–15.4)	7.7 (4.7–15.5)	***0.936***	12.9 (8.3–26.0)	7.5 (5.7–28.9)	***0.821***
Renin	2157.2 (1041.8–2829.0)	357.5 (324.2–407.7)	***<0.001***	1544.2 (812.2–2434.2)	932.1 (285.7–1732.5)	***0.117***	1229.8 (750.6–2396.7)	833.3 (293.9–1677.2)	***0.198***	1402.5 (790.5–2230.4)	876.0 (360.5–1656.2)	***0.110***
AGT	281.9 (245.7–404.8)	21.2 (15.2–32.7)	***<0.001***	309.3 (234.3–449.6)	379.7 (329.0–441.6)	***0.025***	373.6 (294.5–511.8)	435.0 (349.7–591.4)	***0.155***	431.1 (304.0–673.3)	469.4 (390.7–653.1)	***0.380***
ACE	23.2 (17.5–36.0)	12.5 (9.5–15.3)	***<0.001***	21.6 (16.7–33.7)	18.3 (15.9–30.7)	***0.466***	20.2 (16.1–33.4)	19.5 (14.3–28.4)	***0.728***	25.6 (17.5–36.2)	23.0 (16.4–30.2)	***0.281***
ACE2	9.7 (3.9–14.5)	2.1 (1.1–2.8)	***<0.001***	8.6 (5.7–13.7)	7.5 (5.3–9.7)	***0.436***	9.6 (5.6–13.0)	7.9 (5.5–9.9)	***0.160***	9.2 (7.2–15.4)	8.5 (6.4–11.4)	***0.343***
ACE/ACE2	3.6 (1.6–6.9)	6.9 (3.6–13.8)	***0.015***	3.2 (1.4–5.9)	2.9 (1.6–3.5)	***0.647***	2.7 (1.6–5.6)	2.4 (1.7–4.1)	***0.946***	3.1 (1.9–4.2)	2.5 (1.5–4.6)	***0.586***
ANG II	326.4 (203.6–542.9)	1525.5 (559.8–3239.1)	***<0.001***	285.8 (179.6–525.6)	1776.3 (924.1–4471.6)	***<0.001***	334.5 (190.7–586.2)	1845.5 (1152.2–4831.1)	***<0.001***	305.0 (164.7–533.3)	2692.2 (1841.2–5493.96)	***<0.001***
ANG 1-7	210.1 (173.7–450.7)	663.59 (318.0–1247.6)	***<0.001***	202.9 (185.2–303.8)	598.0 (311.8–1179.8)	***<0.001***	223.5 (154.8–382.5)	417.3 (243.0–1159.6)	***0.005***	206.4 (146.0–379.7)	390.8 (264.4–1018.5)	***0.007***
ANG II/ANG 1-7	1.5 (1.1–2.2)	2.1 (1.6–4.0)	***0.002***	1.6 (1.1–1.9)	3.7 (2.8–4.6)	***<0.001***	1.6 (1.0–1.9)	4.4 (4.1–5.7)	***<0.001***	1.4 (1.1–1.6)	4.8 (4.1–8.1)	***<0.001***

SBP is for systolic blood pressure; DBP is for diastolic blood pressure; SV is for stroke volume; CO is for cardiac output; SVR is for systemic vascular resistance; UtA is for uterine arteries; Q is for blood flow volume; PI is for pulsatility index; EFW is for estimated fetal weight; UV is for umbilical vein; sFlt-1 is for soluble fms-like tyrosine kinase 1; PlGF is for placental growth factor; AGT is for angiotensinogen; ACE is for angiotensin-converting enzyme; Ang is for angiotensin.

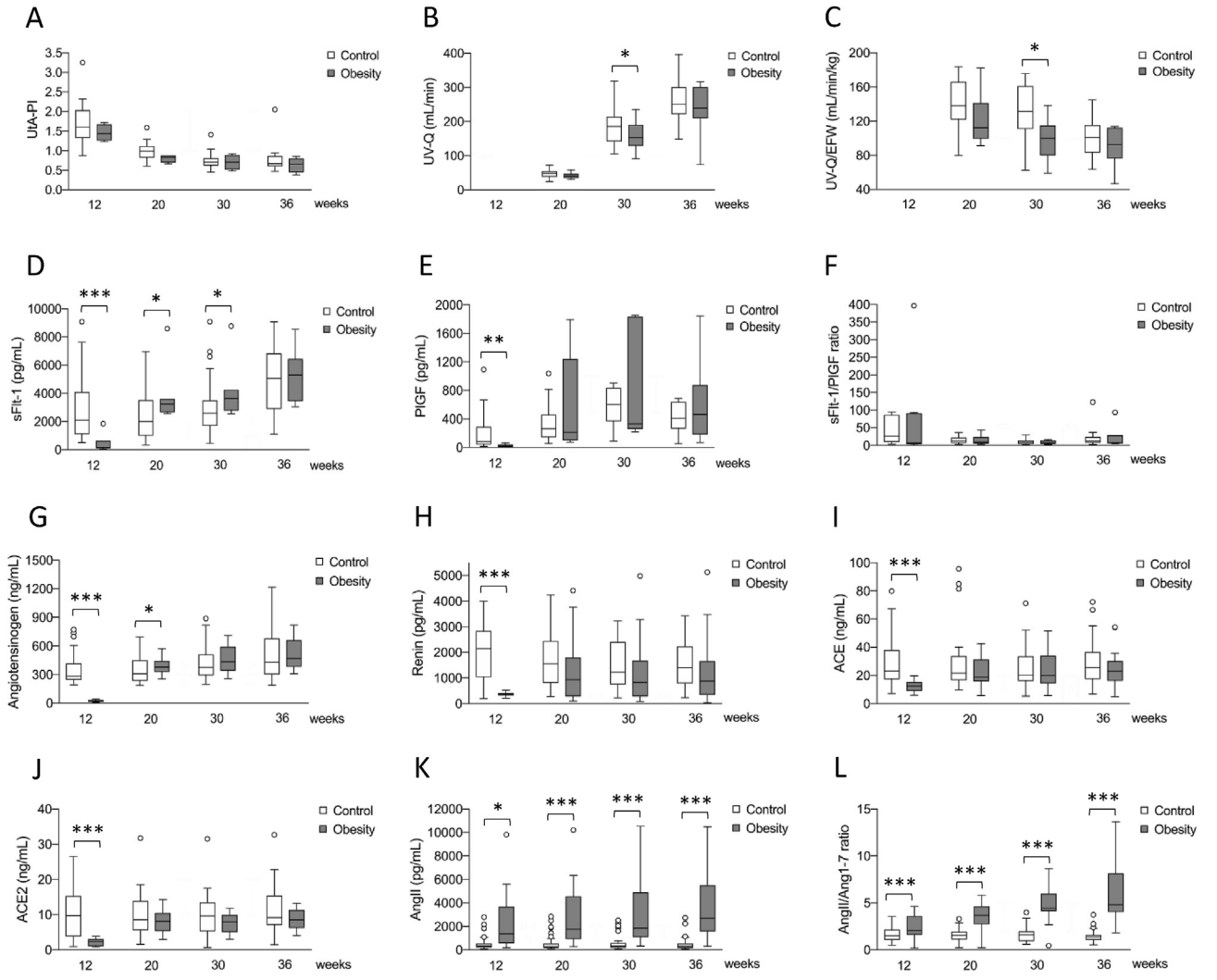

Figure 1 Cross-sectional comparisons of hemodynamic and circulating parameters in obese and control pregnant women (A) Mean uterine artery pulsatility index (UtA-PI); (B) umbilical vein blood flow (UV-Q); (C) umbilical vein blood flow normalized for estimated fetal weight (UV-Q/EFW); (D) (sFlt-1); (E) (PlGF); (F) (sFlt-1/PlGF); (G) Angiotensinogen; (H) Renin; (I) Angiotensin-converting enzyme (ACE); (j) Angiotensin-converting enzyme 2 (ACE2); (K) AngiotensinII (AngII); (L) AngiotensinII/Angiotensin1-7 (AngII/Ang1-7). * is for $p < 0.05$; *** is for $p < 0.001$.

throughout pregnancy (time effect, F 12.6, $p < 0.001$). At 30 weeks of gestation, obese women had significantly lower UV-Q/EFW as compared to controls (103.7 mL/min vs 131.5 mL/min, $p = 0.02$), and they had a greater UV-Q/EFW decrease from week 20–30 (group effect, F 8.0, $p < 0.01$), as shown in Fig. 1C, and Table 3. In other words, obese women showed reduced placental blood supply, as assessed by UV-Q and UV-Q/EFW.

3.3. Circulating angiogenic markers

Obese women displayed significantly lower levels of sFlt-1 at week 12 and 20, but significantly higher levels of sFlt-1 at week 30 as compared to control women (Fig. 1D and Table 2). Throughout pregnancy, sFlt-1 levels increased significantly in both groups (time effect, F 102.2, $p < 0.001$), as shown in Table 3. Nevertheless, in obese women there was a sharp increase between week 12 and 30 (from 151 pg/mL to 3642 pg/mL), while in control women the increase took place between week 30 and 36 (from 2586 pg/mL to 5071 pg/mL), with a resultant interaction effect (F 40.3, $p < 0.001$) (Fig. 1D and Table 3). As for PlGF, it significantly increased in both groups between week 12 and 30 (time effect, F 26.1, $p < 0.001$). The increase was more pronounced in obese women (interaction effect, F 3.5, $p = 0.04$), who had lower basal PlGF levels (19.2 pg/mL) as compared to control women (from 82 pg/mL) (Fig. 1E and Table 3). Notwithstanding these differences, sFlt-1/PlGF did not significantly change throughout pregnancy within and between the groups (Fig. 1F and Table 3).

3.4. Circulating renin-angiotensin system

As for the renin-angiotensin system, in early pregnancy (week 12) obese women had significantly higher levels of circulating AngII and significantly lower levels of circulating angiotensinogen, renin, ACE and ACE2, with a

Table 3 Significances as assessed by longitudinal analysis with Anova-type statistics.

	Variable	Time period (weeks)													
		Total		12 vs 20		12 vs 30		12 vs 36		20 vs 30		20vs36		30vs36	
	Effect	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p
SBP	Group	**2.69**	**0.13**	0.10	0.75	0.14	0.70	3.70	0.06	1.43	0.27	2.74	0.15	2.74	0.15
	Time	**0.68**	**0.52**	0.54	0.46	1.72	0.19	0.20	0.65	0.16	0.68	0.31	0.57	1.77	0.18
	Interaction	**2.08**	**0.12**	2.30	0.13	0.59	0.72	3.74	0.06	0.14	0.71	0.87	0.35	3.70	0.06
DBP	Group	**2.00**	**0.21**	0.59	0.47	2.05	0.15	1.02	0.35	2.35	0.18	1.51	0.27	3.08	0.13
	Time	**0.85**	**0.44**	0.58	0.44	0.10	0.75	1.02	0.31	0.84	0.36	3.31	0.07	0.42	0.52
	Interaction	**1.85**	**0.51**	0.20	0.66	1.14	0.29	0.46	0.50	0.48	0.50	0.001	0.97	2.07	0.15
SV	Group	**0.72**	**0.40**	0.03	0.87	0.02	0.88	0.21	0.64	0.92	0.35	1.56	0.21	1.31	0.25
	Time	**1.85**	**0.14**	2.85	0.09	0.001	0.98	0.40	0.53	3.24	0.07	6.29	0.01	0.30	0.58
	Interaction	**0.96**	**0.41**	2.03	0.15	0.99	0.32	1.84	0.17	0.04	0.85	0.04	0.22	0.18	0.67
CO	Group	**1.61**	**0.22**	1.47	0.24	3.00	0.10	0.92	0.35	1.41	0.25	0.26	0.61	0.52	0.48
	Time	**0.68**	**0.53**	2.33	0.13	1.16	0.28	1.23	0.27	0.01	0.94	0.004	0.95	0.04	0.85
	Interaction	**0.62**	**0.57**	0.97	0.33	0.003	0.95	1.14	0.28	0.15	0.70	0.29	0.58	1.72	0.19
SVR	Group	**0.85**	**0.37**	0.01	0.91	2.26	0.15	0.37	0.55	1.15	0.30	0.03	0.86	1.68	0.21
	Time	**0.80**	**0.47**	1.04	0.31	1.17	0.28	2.15	0.14	0.02	0.90	0.05	0.81	0.04	0.84
	Interaction	**0.90**	**0.42**	0.11	0.74	1.36	0.24	0.01	0.55	2.80	0.09	0.48	0.49	1.68	0.195
UtA-PI	Group	**0.23**	**0.65**	2.18	0.18	0.10	0.77	0.48	0.52	0.19	0.68	0.95	0.38	0.005	0.95
	Time	**50.7**	< **0.001**	139.9	<0.001	216.5	<0.001	161.5	<0.001	19.17	<0.001	15.85	<0.001	0.25	0.617
	Interaction	**0.57**	**0.61**	1.4	0.24	2.4	0.12	0.30	0.58	2.0	0.16	0.54	0.46	0.22	0.65
UtA-Q mean	Group	**0.08**	**0.77**	NA	NA	NA	NA	NA	NA	0.02	0.88	0.17	0.68	0.18	0.67
	Time	**13.81**	< **0.001**	NA	NA	NA	NA	NA	NA	15.89	<0.001	20.39	<0.001	0.01	0.916
	Interaction	**0.76**	**0.46**	NA	NA	NA	NA	NA	NA	0.18	0.669	1.32	0.25	0.85	0.356
UtA-Q mean/EFW	Group	**0.09**	**0.77**	NA	NA	NA	NA	NA	NA	0.77	0.38	0.01	0.91	0.07	0.79
	Time	**59.95**	< **0.001**	NA	NA	NA	NA	NA	NA	50.69	<0.001	90.65	<0.001	17.46	0.916
	Interaction	**0.68**	**0.48**	NA	NA	NA	NA	NA	NA	0.30	0.58	0.92	0.34	0.36	0.55
UV-Q	Group	**5.36**	**0.03**	NA	NA	NA	NA	NA	NA	7.88	0.01	3.66	0.07	4.43	0.08
	Time	**331.8**	< **0.001**	NA	NA	NA	NA	NA	NA	221.01	<0.001	193.9	<0.001	41.89	<0.001
	Interaction	**1.26**	**0.28**	NA	NA	NA	NA	NA	NA	0.03	0.86	0.59	0.44	2.43	0.12
UV-Q/EFW	Group	**8.03**	< **0.01**	NA	NA	NA	NA	NA	NA	8.63	0.007	3.84	0.06	5.26	0.03
	Time	**12.58**	< **0.001**	NA	NA	NA	NA	NA	NA	3.47	0.06	22.89	<0.001	9.04	0.003
	Interaction	**0.83**	**0.43**	NA	NA	NA	NA	NA	NA	0.33	0.57	0.16	0.69	1.54	0.21
sFlt-1	Group	**0.29**	**0.60**	2.18	0.16	2.40	0.14	6.01	0.03	10.97	0.001	3.12	0.11	2.94	0.11
	Time	**102.21**	< **0.001**	411.01	<0.001	199.11	<0.001	173.39	<0.001	9.79	<0.001	36	<0.001	24.96	<0.001
	Interaction	**40.26**	< **0.001**	474.72	<0.001	126.31	<0.001	26.69	<0.001	0.25	0.61	3.45	0.06	3.13	0.08
PlGF	Group	**0.30**	**0.59**	4.01	0.07	6.38	0.02	5.09	0.04	0.05	0.10	0.09	0.76	0.01	0.91
	Time	**26.07**	< **0.001**	28.08	<0.001	38.20	<0.001	29.22	<0.001	18.41	<0.001	2.74	0.10	5.89	0.01
	Interaction	**3.52**	**0.04**	7.44	0.006	4.04	0.04	5.22	0.02	0.83	0.68	0.31	0.58	1.12	0.29
sFlt-1/PlGF	Group	**0.79**	**0.38**	1.15	0.30	1.54	0.23	0.95	0.34	0.02	0.89	0.04	0.84	0.05	0.83
	Time	**1.58**	**0.21**	0.96	0.33	2.43	0.12	0.38	0.53	9.95	0.002	0.65	0.42	8.89	0.003
	Interaction	**0.23**	**0.69**	0.18	0.67	0.29	0.59	0.13	0.72	0.32	0.57	0.003	0.98	0.10	0.75
AGT	Group	**0.11**	**0.73**	13.39	<0.001	14.90	<0.001	15.70	<0.001	3.87	0.06	3.45	0.69	1.55	0.22
	Time	**117.6**	< **0.001**	224.6	<0.001	174.01	<0.001	269.50	<0.001	19.92	<0.001	54.02	<0.001	18.28	<0.001
	Interaction	**44.39**	< **0.001**	210.17	<0.001	74.92	<0.001	78.55	<0.001	0.41	0.52	0.45	0.50	0.10	0.75

(*continued on next page*)

Table 3 (*continued*)

	Variable	Time period (weeks)													
		Total		12 vs 20		12 vs 30		12 vs 36		20 vs 30		20vs36		30vs36	
	Effect	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p	*F*	p
Renin	Group	**10.65**	< **0.01**	34.52	<0.001	30.08	<0.001	33.32	<0.001	2.45	0.13	2.51	0.11	2.00	0.16
	Time	**2.71**	**0.90**	3.74	0.05	1.03	0.31	2.15	0.14	7.4	0.006	3.44	0.06	2.21	0.14
	Interaction	**17.74**	< **0.001**	18.45	<0.001	17.85	<0.001	17.2	<0.001	0.81	0.37	0.24	0.63	0.55	0.46
ACE	Group	**5.92**	**0.02**	15.79	<0.001	15.93	<0.001	19.07	<0.001	0.75	0.39	1.19	0.28	0.84	0.36
	Time	**7.58**	< **0.001**	9.63	0.002	4.79	0.03	15.09	<0.001	0.07	0.78	3.98	0.045	6.38	0.01
	Interaction	**9.08**	< **0.001**	18.95	<0.001	13.40	<0.001	11.59	<0.001	0.07	0.79	0.05	0.82	0.30	0.58
ACE2	Group	**9.23**	< **0.01**	24.71	<0.001	25.56	<0.001	24.92	<0.001	1.54	0.21	1.28	0.26	1.60	0.21
	Time	**31.52**	< **0.001**	45.70	<0.001	46.55	<0.001	68.01	<0.001	0.04	0.84	4.29	0.04	11.31	<0.001
	Interaction	**26.86**	< **0.001**	46.49	<0.001	50.10	<0.001	44.98	<0.001	0.07	0.79	0.02	0.88	0.50	0.48
AngII	Group	**40.41**	< **0001**	28.29	<0.001	31.33	<0.001	36.91	<0.001	40.70	<0.001	47.80	<0.001	52.23	<0.001
	Time	**4.28**	**0.01**	2.29	0.01	8.11	0.004	5.03	0.02	2.70	<0.08	2.70	0.10	0.25	0.62
	Interaction	**8.34**	< **0.001**	6.39	0.001	6.91	0.009	13.69	<0.001	4.78	0.537	4.78	0.03	7.41	0.006
Ang1-7	Group	**11.84**	< **0.001**	15.92	<0.001	12.73	<0.001	13.20	<0.001	9.82	0.002	10.37	0.001	7.93	0.005
	Time	**4.59**	< **0.01**	1.80	0.18	5.69	0.02	8.37	0.004	4.04	0.04	4.85	0.03	0.37	0.54
	Interaction	**2.25**	**0.09**	0.84	0.36	3.09	0.08	3.70	0.05	2.46	0.12	2.27	0.13	0.002	0.97
AngII/Ang1-7	Group	**59.28**	< **0.001**	26.20	<0.001	32.00	<0.001	57.49	<0.001	52.52	<0.001	91.97	<0.001	89.22	<0.001
	Time	**10.70**	< **0.001**	13.66	<0.001	16.35	<0.001	12.18	<0.001	7.15	0.01	2.57	0.11	0.06	0.81
	Interaction	**17.00**	< **0.001**	17.26	<0.001	15.58	<0.001	29.32	<0.001	3.41	0.06	13.18	<0.001	5.99	0.01

SBP is for systolic blood pressure; DBP is for diastolic blood pressure; SV is for stroke volume; CO is for cardiac output; SVR is for systemic vascular resistance; UtA is for uterine arteries; Q is for blood flow volume; PI is for pulsatility index; EFW is for estimated fetal weight; UV is for umbilical vein; sFlt-1 is for soluble fms-like tyrosine kinase 1; PlGF is for placental growth factor; AGT is for angiotensinogen; ACE is for angiotensin-converting enzyme; Ang is for angiotensin.

resultant significantly higher AngII/Ang1-7 ratio (Fig. 1G– L and Table 2). Throughout pregnancy, angiotensinogen increased over time in both groups (time effect, F 117.6, $p < 0.001$), but the increase between week 12 and 36 was significantly higher in obese women (interaction effect, F 44.4, $p < 0.001$) (Fig. 1G and Table 3). Renin changed in an opposite direction between the groups over time, as it was 2157 pg/mL at week 12 and 1402 pg/mL at week 36 in control women, while it was 357 pg/mL at week 12 and 876 pg/mL at week 36 in obese women (interaction effect, F 17.7, $p < 0.001$) (Fig. 1H and Table 3). ACE and ACE2 levels did not change in control women, while they increased from the week 12–36 in obese women (Fig. 1I and J). As a result, AngII, which was already significantly higher in obese women at week 12, continued to increase throughout pregnancy (from 1525 pg/mL at week 12 to 2692 pg/mL at week 36). By contrast, AngII remained unchanged in control women, being 326 pg/mL at week 12 and 305 pg/mL at week 36 (interaction effect F 8.3, $p < 0.001$) (Fig. 1K and Table 3). In line with these changes, obese women displayed significantly higher levels of the ratio AngII/Ang1-7, which progressively increased during the whole pregnancy (Fig. 1L and Table 3).

3.5. Correlation coefficients and linear regression

Placental blood supply, as assessed by UV-Q and UV-Q/EFW at week 30 showed a significant inverse correlation with AngII and AngII/Ang1-7 (Table 4). In addition, women with GDM had significantly lower UV-Q (146.68 ± 42.55 vs 188.2 ± 48.91, $p < 0.01$) and lower UV-Q/EFW (91.03 ± 26.66 vs 117.99 ± 26.57, $p < 0.01$), as compared to pregnant women with no GDM. The linear regression analysis confirmed the presence of an independent association between AngII/Ang1-7 and UV-Q at week 30, as well as between GDM and UV-Q/EFW at week 30 (Table 5). Interestingly, UV-Q at week 30 was associated with newborn body weight ($p = 0.02$; rho = 0.34), while UV-Q/EFW was not ($p = 0.08$; rho = 0.26).

Table 4 Correlation coefficients.

Variable	UV-Q		UV-Q/EFW	
	rho	p-value	rho	p-value
Age	−0.04	0.79	−0.06	0.70
BMI baseline	−0.22	0.13	−0.26	0.07
BMI 30th weeks	−0.22	0.13	−0.26	0.07
sFLT-1/PlGF	0.24	0.09	0.29	0.04
AngII	−0.31	0.03	−0.33	0.02
AngII/Ang1-7	−0.40	0.004	−0.41	0.003

Table 5 Linear regression model.

Predictive variables	Dependent variable: UV-Q			
	B-estimate	95 % CI	Standard error	p-value
Age	−0.80	[-3.93, 2.32]	1.55	0.61
BMI baseline	1.63	[-1.80, 5.07]	1.70	0.34
GDM	−28.68	[-61.47, 4.11]	16.23	0.08
sFlt-1/PlGF	0.82	[0.16, 1.47]	0.33	0.02
AngII/Ang1-7	−9.37	[-18.26, −0.49]	4.40	0.04
Dependent variable: UV-Q/EFW				
Predictive variables	B-estimate	95 % CI	Standard error	p-value
Age	−0.62	[-2.48, 1.24]	0.92	0.50
BMI baseline	0.65	[-1.40, 2.69]	1.01	0.52
GDM	−19.93	[-39.44, −0.41]	9.66	0.05
sFlt-1/PlGF	0.36	[-0.03, 0.76]	0.20	0.07
AngII/Ang1-7	−4.64	[-9.97, 0.65]	2.62	0.08

4. Discussion

First of all, the present study shows that in obese pregnant women, who had a higher prevalence of GDM but were normotensive, placental blood supply, as assessed by umbilical vein blood flow (UV-Q), was lower as compared to control pregnant women, also when it was adjusted for estimated fetal weight (UV-Q/EFW). This difference reached statistical significance at 30 weeks of gestation.

Obesity affects placental blood supply. Animal models of high-fat-induced obesity exhibited reduced placental blood flow volume associated with increased placental infarction and calcification [21]. In uncomplicated pregnancies of obese women, there was a higher rate of maternal placental vascular lesions as compared to normal weight controls (46.8 % vs 28.2 %) [9], and fetuses were more hypoxic as compared to normal weight women, suggesting that obesity can affect fetal oxygenation [6]. Consistent with these findings, our study shows that at week 30 of gestation, obese women had significant lower placental blood supply, as assessed by UV-Q and UV-Q/EFW, with no differences in uterine artery resistance. This is in line also with a recent study reporting that in a group of overweight women (BMI = 28) with polycystic ovary syndrome, the umbilical blood flow from the placenta to the fetus was impaired as compared to a low-risk reference population (BMI = 23.4), although the placental resistance was not increased, i.e. comparable pulsatility index in the uterine artery [22].

Adequate placental blood supply is essential to support fetal growth [23] and reduction of umbilical vein blood flow is an early sign of growth restriction [24,25]. Consistent with this concept, in the present study, we found that UV-Q at week 30 of gestation was related to the newborn body weight. Previous studies have shown that blood flow is reduced in early and late fetal growth restriction [26], and we have recently demonstrated that UV-Q and UV-Q/EFW were lower in late-term fetuses that experienced a growth drop and/or with cerebral blood flow redistribution, even if their biometric percentiles were within normal ranges [18]. In addition, the fetuses delivered by emergency operative delivery during labor due to non-reassuring fetal status had a significantly lower UV-Q [18]. Consistent with these observations, fetuses with UV-Q below the 20th percentile seem to be at increased risk of intrapartum fetal compromise [27,28].

Most importantly, as this was the aim of the present study, our data shows for the first time that normotensive

obese pregnant women had significantly higher levels of circulating AngII and AngII/Ang1-7 ratio, indicating RAS dysregulation. In addition, placental blood supply, as assessed by UV-Q and UV-Q/EFW, was inversely related to AngII and AngII/Ang1-7 levels. Interestingly, the association between UV-Q and AngII/Ang1-7 levels was independent from age, BMI, GDM, as well as sFlt-1/PlGF ratio.

It is well known that the renin-angiotensin system (RAS) is upregulated during pregnancy, and the fetal-placental unit is an important additional site of RAS activity [7]. Pregnancy is associated with an increase of angiotensinogen [29], which is stimulated not only by high circulating levels of estrogens [30], but also by ACTH and cortisol [29]. Our data confirm angiotensinogen increase throughout pregnancy, but they show that obese pregnant women had RAS dysregulation. In particular, obese pregnant women, who displayed higher AngII levels at week 12, showed a further progressive significant increase of AngII levels throughout gestation as compared to control pregnant women, whose AngII levels did not increase. Likewise, its product Ang1-7, the ratio AngII/Ang1-7 increased progressively in obese women while in control pregnant women remained unchanged.

The fact that obese pregnant women at week 12 displayed higher levels of AngII, but lower of angiotensinogen and renin as compared to controls, is likely to be due to an upregulation of tissue RAS activity in extra-renal sites such as the adipose tissue and/or the placenta, with subsequent higher AngII levels and inhibition of angiotensinogen and renin [4]. Then, throughout gestation, renin, ACE, and ACE2 increased in obese but not in normal weight woman. As a result, obese women, who displayed higher AngII levels at week 12, showed a further significant progressive increase of AngII levels, and the ratio AngII/Ang1-7 during gestation. Another possible mechanism underlying AngII increase is that maternal overweight reduces the expression of neprilysin in the feto-placental endothelium, which may alter the balance of vasoactive peptides [11].

Interestingly, notwithstanding AngII and AngII/Ang1-7 increase, obese women remained normotensive, possibly due to the well-known resistance to the pressor effect of AngII of pregnant women [31]. This resistance has been attributed to: (i) the natriuretic effect of progesterone; (ii) the relaxing effect of progesterone on vascular smooth muscle cells; (iii) the shunting of blood to the pelvic viscera; and (iv) the relative physiologic undefilling until approximately 30 weeks of gestation [31]. It has been recently underlined that it remains unknown whether RAS dysregulation is a prerequisite for the development of hypertensive disorders of pregnancy or a consequence [5]. Our data suggest that high levels of AngII are a prerequisite and might predispose obese women to further hemodynamic changes, when the resistance to AngII is lost [31], such as in women with gestational hypertensive disorders [32]. For instance, administration of AngII in pregnant stroke-prone spontaneously hypertensive rats significantly impact maternal cardiovascular physiology and fetal development, leading to blood pressure increase, uterine artery remodeling, placental dysfunction, and fetal growth restriction [33].

The angiogenic factors sFlt-1 and PlGF are considered maternal biomarkers of defective angiogenesis, and many current guidelines recommend measuring the sFlt-1/PlGF ratio to help in the diagnosis of pre-eclampsia [13]. Our data show that the ratio sFlt-1/PlGF did not change between obese and control women throughout pregnancy. Nevertheless, obesity was associated with significant changes of the circulating levels of both. For instance, obese women had significantly lower levels of sFlt-1 and PlGF at week 12, and a sharp increase of both from week 12 to week 36 as compared to control women. These findings are consistent with several studies reporting that obesity was associated with lower sFlt-1 and PlGF levels in women with and without placenta-related disorders [34–37]. It has been argued that relatively low sFlt-1 may reflect a predisposition to placental pathology in obese women, regardless of pregnancy outcome [35]. As suggested by other Authors [34], these differences should be taken into account when these markers are used separately and not as a ratio in pre-eclampsia prediction in obese women.

Finally, we discuss the strengths and limitations of this study. Our study has several strengths. In particular, its prospective design allowed us to schedule a clinical and a maternal hemodynamic assessment with blood sampling at week 12, 20, 30, and 36 of gestation. We also scheduled three ultrasounds to measure fetal biometry, feto-placental Doppler velocimetry, uterine artery and umbilical vein blood flow at week 20, 30, and 36 of gestation. It has to be noted that although the umbilical vein blood flow offers many useful information it is still used mostly in research settings [24]. To study the RAS, we looked not only at AngII but also at relatively new RAS components such as ACE2 and Ang1-7 (which have been implicated in growth regulation [8,38] and we have recently found decreased ACE2 in children with short stature [39]). On the other hand, we acknowledge that the study has some limitations, as we did not measure blood pressure with 24-h ambulatory blood pressure monitoring [40], and we did not check for tissue ACE, ACE2 and angiotensins, nor enzyme activity in placentas or umbilical cord samples. In addition, the study was powered to detect a mean difference in AngII levels, which might explain the lack of statistical significance for some hemodynamic and ultrasound variables at certain time points.

In conclusion, our study shows for the first time that normotensive obese women exhibited a significant progressive increase of AngII and AngII/Ang1-7 throughout pregnancy, indicating RAS dysregulation, which was inversely related to placental blood supply. This was assessed by umbilical vein blood flow (UV-Q) and umbilical blood flow adjusted for estimated fetal weight (UV-Q/EFW) and obese pregnant women exhibited a significant reduction of both at 30 weeks of gestation. Our data shed light on early changes in pregnant obese women, and suggest that RAS dysregulation is a prerequisite rather

than a consequence of hypertensive disorders of pregnancy and other maternal neonatal complications.

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Declaration of competing interest

The authors declare no conflict of interest.

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