Circulating Fatty Acid Synthase in pregnant women: Relationship to blood pressure, maternal metabolism and newborn parameters

Gemma Carreras-Badosa1,2, Anna Prats-Puig1,2, Teresa Puig3, Montserrat Vázquez-Ruíz4, Monserrat Bruel5, Ericka Mendoza5, Francis de Zegher6, Lourdes Ibáñez7,8, Abel López-Bermejo1,2 & Judit Bassols1,2

The enzyme FASN (fatty acid synthase) is potentially related with hypertension and metabolic dysfunction. FASN is highly expressed in the human placenta. We aimed to investigate the relationship circulating FASN has with blood pressure, maternal metabolism and newborn parameters in healthy pregnant women. Circulating FASN was assessed in 115 asymptomatic pregnant women in the second trimester of gestation along with C-peptide, fasting glucose and insulin, post-load glucose lipids, HMW-adiponectin and blood pressure (the latter was assessed in each trimester of gestation). At birth, newborns and placentas were weighed. FASN expression was also able to be assessed in 80 placentas. Higher circulating FASN was associated with lower systolic blood pressure (SBP), with a more favourable metabolic phenotype (lower fasting glucose and insulin, post load glucose, HbAc1, HOMA-IR and C-peptide), and with lower placental and birth weight (all p < 0.05 to p < 0.001). Placental FASN expression related positively to circulating FASN (p < 0.005) and negatively to placental weight (p < 0.05). Our observations suggest a physiological role of placental FASN in human pregnancy. Future studies will clarify whether circulating FASN of placental origin does actually regulate placental and fetal growth, and (thereby) has a favourable influence on the pregnant mother’s insulin sensitivity and blood pressure.

The multifunctional protein complex FASN (fatty acid synthase) is indispensable in the synthesis of saturated straight-chain fatty acids from acetyl coenzyme A (CoA), via malonyl-CoA1. Excess energy intake and increased insulin levels has the effect of upregulating the FASN gene expression2,3; suggesting this enzyme is implicated in energy homeostasis.

Altered FASN activity/expression has been reported in metabolic syndrome and overweight subjects who exhibit obesity, inflammation, hypertension, insulin resistance, dyslipidemia and atherosclerosis, indicating a relationship between FASN and the pathogenesis of hypertension and metabolic dysfunction4,5. Adipose tissue from hypertensive individuals showed decreased levels of FASN mRNA6. The subcutaneous adipose tissue of the obese subjects also showed decreased FASN expression compared to lean subjects7–11, and has exhibited negative correlation with insulin resistance markers such as glucose, HbA1c and HOMA-IR6–8. In adipose tissue of insulin

1Pediatrics, Girona Institute for Biomedical Research, 17007 Girona, Spain. 2Pediatrics, Dr. Josep Trueta Hospital, 17007 Girona, Spain. 3TargetsLab, Medical Sciences Department, Faculty of Medicine, University of Girona, 17003 Girona, Spain. 4Pediatrics, Salut Empordà Foundation, 17600 Figueres, Spain. 5Obstetrics and Gynecology, Salut Empordà Foundation, 17600 Figueres, Spain. 6Department of Development & Regeneration, University of Leuven, 3000 Leuven, Belgium. 7Pediatric Endocrinology Unit, Sant Joan de Déu Children’s Hospital, 08950 Esplugues, Barcelona. 8CIBERDEM (Center for Network Biomedical Research in Diabetes and Related Metabolic Diseases), ISCIII, Madrid, Spain. Correspondence and requests for materials should be addressed to A.L.-B. (email: alopezbermejo@idibgi.org) or J.B. (email: jbassols@idibgi.org)
resistant type 2 diabetic patients, FASN mRNA expression is markedly decreased in response to reduced insulin signalling\(^2\).

**In vivo**, the cellular concentrations of poly-unsaturated fatty acids and sterols are the main factors regulating the expression of FASN, when their concentrations decrease in the cell, the transcription of the FASN gene increases. FASN can be actively removed out of the cell when AMPK (adenosine monophosphate-activated protein kinase) is activated\(^9\). Circulating FASN is thus thought to reflect previous intracellular enzymatic activity\(^9\).

In normal cells, low levels of FASN are present due to abundant dietary lipids. However, FASN is highly expressed in hepatic, adipose tissue and in neoplastic cells, where FASN expression and the synthesis of new fatty acids are up-regulated as a survival advantage to low-fuel supply\(^13,14\). The placenta also expresses high amounts of FASN\(^15,16\). Trophoblastic cells may use *de novo* lipid synthesis in order to maintain essential placental actions for development. This strategy may also be an evolutionary favoured compensatory mechanism, as the lipid supply from food intake may become limited during pregnancy.

The role of FASN in human pregnancy is poorly studied. A recent report indicates that maternal obesity and gestational diabetes are related to less expression of FASN in adipose tissue of subcutaneous and visceral origin\(^17\). In mice with a lipid-poor diet during gestation, an augmented expression of FASN in adipose tissue was reported\(^18\).

Despite the core physiological role of FASN in maintaining normal levels of lipids and glucose, as well as energy homeostasis and its high expression in placenta, the relationship between the circulating form of this molecule, blood pressure and metabolism during human pregnancy has not been characterized. In this work, we studied the associations of circulating FASN with blood pressure, maternal metabolism and newborn parameters in normal human pregnancy. We also studied whether circulating FASN was related to placental FASN expression.

**Results**

Table 1 summarizes the clinical and laboratory findings of the study subjects.

**Correlation analyses.** Second- and third-trimester SBP decreased with increasing circulating FASN. Higher circulating FASN was also related to a more favourable metabolic condition, specifically lower fasting glucose and insulin, post load glucose, HbAc1, HOMA-IR and C-peptide (all \(p < 0.05\) to \(p < 0.001\); Table 2). Circulating FASN was inversely related to placental weight and birth weight (\(p < 0.05\) to \(p < 0.01\); Table 2), and directly associated with placental FASN mRNA expression (\(p < 0.001\); Table 2). Placental FASN expression was also inversely related

| Clinical assessments | 1st Trimester | 2nd Trimester | 3rd Trimester |
|---------------------|--------------|--------------|--------------|
| n                   | 115          | 115          | 115          |
| Age (yr)            | 30.2 ± 0.2   | –            | –            |
| Height (m)          | 163 ± 1      | –            | –            |
| Weight (Kg)         | 66 ± 1       | 72 ± 1       | 77 ± 1       |
| BMI (Kg/m\(^2\))    | 25 ± 1       | 27 ± 1       | 29 ± 1       |
| SBP (mm Hg)         | 116 ± 1      | 117 ± 1      | 119 ± 1      |
| DBP (mm Hg)         | 68 ± 1       | 69 ± 1       | 72 ± 1       |
| Laboratory variables|              |              |              |
| Pre-load glucose (mg/dL) | –       | 78 ± 1     | –            |
| Post-load glucose (mg/dL) | –       | 113 ± 3    | –            |
| HbA1C (%)           | –            | 5.0 ± 0.1   | –            |
| Fasting insulin (μIU/mL) | –       | 6.0 ± 0.5  | –            |
| HOMA-IR             | –            | 1.2 ± 0.1   | –            |
| C-peptide           | –            | 1.6 ± 0.1   | –            |
| HMW-adiponectin (mg/L) | –       | 6.1 ± 0.2  | –            |
| Triacylglycerol (mg/dL) | –       | 161 ± 1    | –            |
| Total cholesterol (mg/dL) | –       | 257 ± 2    | –            |
| HDL cholesterol (mg/dL) | –       | 71 ± 1     | –            |
| LDL cholesterol (mg/dL) | –       | 154 ± 2    | –            |
| Circulating FASN (ng/mL) | –       | 4.4 ± 0.2  | –            |
| Placental and Newborns’ Parameters |              |              |              |
| Placental FASN expression\(^a\) | –       | –          | 0.8 ± 0.1   |
| Placental weight (g) | –            | –          | 603 ± 8     |
| Birth weight (g)    | –            | –          | 3300 ± 30   |
| Birth length (cm)   | –            | –          | 49.5 ± 0.1  |
| Birth weight SDS    | –            | –          | 0.1 ± 0.1   |
| Birth length SDS    | –            | –          | −0.1 ± 0.1  |

\(^a\)Assessed after parturition in 80 women.

Table 1. Clinical and laboratory assessments in healthy pregnant women. Data are shown as mean ± SEM. BMI: body mass index; SBP & DBP: systolic and diastolic blood pressure; HOMA-IR: homeostasis model assessment insulin resistance; FASN: fatty acid synthase.
to the weight of the placenta (p < 0.05), however, it was unrelated to blood pressure or to metabolic parameters in pregnant women (data not shown).

**Tertiles of circulating FASN.** A threshold association was evident for circulating FASN and BP, metabolic variables and the parameters of newborns, with women in the highest tertile of circulating FASN exhibiting lower SBP in the second and third trimester of gestation, DBP in the third trimester, fasting glucose and insulin, post load glucose HOMA-IR, placental FASN expression, placental weight and birth weight SDS, and higher HMW-adiponectin compared to other tertiles of circulating FASN (Table 3 and Fig. 1 and Suppl Fig. 1).

**Multiple regression analyses.** In multiple regression analyses (Table 4), circulating FASN remained independently related to the second- (β = −0.231, p = 0.008) and third- (β = −0.333, p < 0.001) trimester SBP, fasting and post-load glucose (β = −0.204, p = 0.028 and β = −0.261, p = 0.004, respectively), HOMA-IR (β = −0.257, p = 0.006), and placental and birth weight (β = −0.214, p = 0.030 and β = −0.194, p = 0.015, respectively), even when confounding variables where introduced in the model.

Placental FASN expression also remained correlated to placental weight (β = −0.201, p < 0.05) and birth weight SDS (β = −0.247*, p < 0.01 and ***p < 0.005 from Pearson correlations.

**Discussion**

Higher circulating FASN was associated with a more favourable blood pressure and metabolic profile, with lower placental weight and birth weight and with higher placental FASN expression in healthy pregnant women.

The independent associations of circulating FASN with blood pressure and with glucose- and insulin-related markers concur with previous reports in overweight and hypertensive subjects that showed decreased FASN expression in their fat depots. Additionally, a negative relation between FASN expression and insulin resistance markers, has been reported in adipose tissue of healthy subjects without diabetes. Evidence, however, exists for an increased FASN expression and higher circulating FASN in obesity-related disorders. These discrepancies may be explained, at least in part, by the apparently opposing effects of insulin on FASN, causing both long-term upregulation of FASN expression and short-term FASN inactivation; the latter being dependent on the insulinemic state of the individual. Our study was based on healthy pregnant women with no comorbidities. These associations may vary in patients with chronic insulin resistance including patients with vascular or metabolic comorbidities and long-standing obesity.

| Clinical assessments | 1st Trimester | 2nd Trimester | 3rd Trimester |
|----------------------|--------------|--------------|--------------|
| Age (yr)             | 0.020        |              |              |
| Height (m)           | −0.162       |              |              |
| Weight (Kg)          | −0.149       | −0.142       | −0.137       |
| BMI (Kg/m²)          | −0.085       | −0.069       | −0.062       |
| SBP (mm Hg)          | −0.107       | −0.256**     | −0.280**     |
| DBP (mm Hg)          | −0.175       | −0.116       | −0.210*      |

**Laboratory variables**

| Pre-load glucose (mg/dL) | −              | −0.227**      |              |
| Post-load glucose (mg/dL) | −              | −0.298***     |              |
| HbA1C (%)                | −              | −0.225*       |              |
| Fasting insulin (μIU/ml) | −              | −0.274**      |              |
| HOMA-IR                  | −              | −0.285**      |              |
| C-peptide (ng/mL)        | −              | −0.210*       |              |
| HMW-adiponectin (mg/L)   |              | 0.144         |              |
| Triacylglycerol (mg/dL)  |              | 0.011         |              |
| Total cholesterol (mg/dL) | −             | 0.016         |              |
| HDL cholesterol (mg/dL)  | −              | −0.037        |              |
| LDL cholesterol (mg/dL)  | −              | 0.020         |              |

**Placental and Newborns' Parameters**

| Placental FASN expression | −              |              | 0.348***     |
| Placental weight (g)      | −              |              | −0.201*      |
| Birth weight (g)          | −              |              | −0.194*      |
| Birth length (cm)         | −              |              | −0.151       |
| Birth weight SDS          | −              |              | −0.247**     |
| Birth length SDS          | −              |              | −0.174       |

Table 2. Correlation analyses of circulating FASN with clinical and laboratory parameters in healthy pregnant women. BMI: body mass index; SBP & DBP: systolic and diastolic blood pressure; HOMA-IR: homeostasis model assessment insulin resistance; FASN: fatty acid synthase. *Assessed after parturition in 80 women. *p < 0.05, **p < 0.01 and ***p < 0.005 from Pearson correlations.
rather than mRNA levels with energy metabolism in healthy pregnant women. Our present results suggest a better correlation of protein FASN expression in human placenta is influenced by maternal cholesterolemia and glycemia. We do not exclude the possibility that mRNA FASN levels may be also associated with metabolic parameters in pregnant women with comorbidities, such as gestational diabetes, hypertension or dyslipidemia. Our present results suggest a better correlation of protein FASN activity with energy metabolism compared to mRNA levels.

Although the metabolic regulation of FASN is not yet completely understood, these current results suggest that circulating FASN may be among the factors integrating blood pressure regulation and energy metabolism during gestation. AMPK may provide a plausible molecular mechanism for these associations. Into the cell, FASN activity is limited by the activation of the nuclear AMPK enzyme that removes FASN from the cytosolic milieu during gestation. AMPK may provide a plausible molecular mechanism for these associations. Into the cell, FASN provides trophoblasts with an alternative mechanism to maintain a regular supply of fatty acids to keep up with the metabolic demands of this organ.

Preeclampsia is a systemic disorder of pregnancy originating in the placenta. Research has demonstrated that several placental antiangiogenic factors are liberated into circulation during pregnancy and cause widespread endothelial dysfunction, hypertension and other systemic manifestations of preeclampsia. Besides adipose tissue, FASN is highly expressed in normal trophoblastic cells. FASN provides trophoblasts with an alternative mechanism to maintain a regular supply of fatty acids to keep up with the metabolic demands of this organ. Changes in FASN activity may thus cause trophoblastic cell dysfunction which may, in turn, affect the regulation of blood pressure and energy metabolism during gestation. Recent data indicate that FASN expression in human placenta is influenced by maternal cholesterol and glycemia. We do not exclude the possibility that mRNA FASN levels may be also associated with metabolic parameters in pregnant women with comorbidities, such as gestational diabetes, hypertension or dyslipidemia. Our present results suggest a better correlation of protein FASN activity with energy metabolism in healthy pregnant women.

### Table 3. Clinical and laboratory assessments in healthy pregnant women according to tertiles of circulating FASN

| Clinical assessments | Circulating FASN tertiles | 0.1–1.4 ng/ml | 1.4–4.3 ng/ml | 4.5–18.8 ng/ml |
|---------------------|--------------------------|---------------|---------------|----------------|
| n                   |                          | 38            | 39            | 38             |
| Age (yr)            |                          | 30 ± 1        | 29 ± 1        | 31 ± 1         |
| Height (m)          |                          | 163 ± 1       | 163 ± 1       | 162 ± 1        |
| 1st trimester       |                          |               |               |                |
| BMI (Kg/m²)         |                          | 26 ± 1        | 25 ± 1        | 24 ± 1         |
| SBP (mm Hg)         |                          | 118 ± 2       | 119 ± 2       | 114 ± 2        |
| DBP (mm Hg)         |                          | 71 ± 1        | 70 ± 1        | 67 ± 2         |
| 2nd trimester       |                          |               |               |                |
| BMI (Kg/m²)         |                          | 28 ± 1        | 27 ± 1        | 26 ± 1         |
| SBP (mm Hg)         |                          | 119 ± 2       | 120 ± 1       | 114 ± 2*       |
| DBP (mm Hg)         |                          | 70 ± 1        | 69 ± 2        | 68 ± 1         |
| 3rd trimester       |                          |               |               |                |
| BMI (Kg/m²)         |                          | 30 ± 1        | 29 ± 1        | 28 ± 1         |
| SBP (mm Hg)         |                          | 123 ± 2       | 121 ± 2       | 116 ± 2**      |
| DBP (mm Hg)         |                          | 75 ± 1        | 72 ± 1        | 70 ± 2*        |
| Laboratory variables|                          |               |               |                |
| Pre-load glucose (mg/dL) |                        | 80 ± 1        | 80 ± 1        | 76 ± 1*        |
| Post-load glucose (mg/dL) |                        | 119 ± 5       | 122 ± 4       | 101 ± 5**      |
| HbA1C (%)           |                          | 5.1 ± 0.1     | 5.0 ± 0.1     | 4.9 ± 0.1      |
| Fasting insulin (μIU/mL) |                       | 6.8 ± 0.8     | 5.9 ± 0.5     | 4.4 ± 0.7**    |
| HOMA-IR             |                          | 1.3 ± 0.2     | 1.1 ± 0.1     | 0.8 ± 0.1**    |
| C-peptide           |                          | 1.6 ± 0.1     | 1.6 ± 0.1     | 1.4 ± 0.1      |
| HMW-adiponectin (g/L) |                        | 5.8 ± 0.4     | 4.9 ± 0.3     | 6.8 ± 0.5**    |
| Triacylglycerol (mg/dL) |                        | 160 ± 11      | 175 ± 9       | 153 ± 8        |
| Total cholesterol (mg/dL) |                       | 251 ± 8       | 268 ± 8       | 254 ± 9        |
| HDL cholesterol (mg/dL) |                        | 68 ± 2        | 74 ± 2        | 69 ± 3         |
| LDL cholesterol (mg/dL) |                        | 151 ± 7       | 160 ± 7       | 153 ± 7        |
| Circulating FASN (ng/mL) |                      | 0.7 ± 0.1     | 2.8 ± 0.1     | 10.1 ± 1***    |
| Placental and Newborn’s Parameters |                  |               |               |                |
| Placental FASN expression |                | 0.6 ± 0.1     | 0.7 ± 0.1     | 1.1 ± 0.1***   |
| Placental weight (g) |                        | 625 ± 25      | 593 ± 20      | 585 ± 23*      |
| Birth weight (g)    |                        | 3382 ± 53     | 3272 ± 50     | 3256 ± 47      |
| Birth length (cm)   |                        | 50 ± 0.3      | 50 ± 0.2      | 49 ± 0.2       |
| Birth weight SDS    |                        | 0.2 ± 0.1     | 0.1 ± 0.1     | −0.1 ± 0.1*    |
| Birth length SDS    |                        | −0.1 ± 0.2    | −0.1 ± 0.2    | −0.3 ± 0.1     |

*Assessed after parturition in 80 women. *p < 0.05, **p < 0.01 and ***p < 0.001 from One-Way ANOVA.
Our results showed negative associations of placental weight and/or birth weight with circulating FASN and placental FASN expression. The human placenta is comprised by a trophoblast layer that physically limits maternal and fetal blood flows. Maternal plasma lipoproteins cross the placenta to supply the fetus with lipids. Figure 1. Distribution of metabolic parameters: blood pressure (second- and third-trimester systolic blood pressure (SBP)), HMW-adiponectin, pre- and post-load glucose, insulin, HOMA-IR and placental FASN expression according to tertiles of circulating FASN (1st: 0.1–1.4 ng/ml; 2nd: 1.4–4.3 ng/ml and 3rd: 4.5–18.8 ng/ml). Data are means and SEM.

(a)

| SBP (mmHg) | 2nd Trimester | 3rd Trimester |
|------------|---------------|---------------|
| BMI (Kg/m²) | 0.373         | 0.258         |
| FASN (ng/ml) | −0.231        | −0.333        |

(b)

| Metabolic Variables | Pre-load Glucose | Post-load Glucose | HOMA-IR |
|---------------------|-----------------|-----------------|---------|
| BMI (Kg/m²)         | 0.257           | 0.200           |
| FASN (ng/ml)        | −0.204          | −0.261          |
| HOMA-IR (uIU/ml)    | 0.187           | −0.257          |

(c)

| Newborns’ Parameters | Placental Weight | Birth Weight |
|----------------------|-----------------|--------------|
| BMI (Kg/m²)          | 0.247           | −              |
| FASN (ng/ml)         | −0.214          | −0.194        |
| Gestational age      | 0.219           | 0.610         |

Table 4. Multivariate linear models of circulating FASN and gestational SBP, metabolic variables and newborn’s parameters in healthy pregnant women. (a) Non-predictive variables: age, HbA1c, HOMA-IR and serum lipids. (b) Non-predictive variables: age and serum lipids. (c) Non-predictive variables: sex, maternal age, pre- or post-load glucose, HbA1c, HOMA-IR and serum lipids.

| Placental FASN expression | Placental Weight |
|---------------------------|-----------------|
| Beta                      | −0.238          |
| Sig.                      | 0.047           |
| R²                        | 5.6%            |

| Gestational age | Placental Weight |
|-----------------|-----------------|
| Beta            | 0.323           |
| Sig.            | 0.008           |
| R²              | 10.6%           |

Table 5. Multivariate linear models of placental FASN expression and placental weight in healthy pregnant women. Non-predictive variables: sex, maternal age and BMI, pre- or post-load glucose, HbA1c, HOMA-IR and serum lipids. *Assessed after parturition in 80 women.

Our results showed negative associations of placental weight and/or birth weight with circulating FASN and placental FASN expression. The human placenta is comprised by a trophoblast layer that physically limits maternal and fetal blood flows. Maternal plasma lipoproteins cross the placenta to supply the fetus with lipids. Fetal
tissues and the placenta also synthetize fatty acids and cholesterol to compensate for a possible lipid deficiency during pregnancy24. Therefore, we would suggest that the changes in FASN expression in smaller placentas may be a compensatory mechanism to regulate maternal blood pressure and metabolic parameters in normal pregnancies with lower fetoplacental growth.

Increased FASN expression may regulate the fatty acid outflow from other tissues, such as adipose tissue, preventing placentas from being too big or too small, as well as contributing to an improvement in the physiological insulin-resistant state that is observed during pregnancy. In this sense, during pregnancy, to compensate for a poor lipid diet, several adaptations of the maternal metabolism may be the increasing expression of those genes implicated in the synthesis of fatty acids (among them FASN) in the maternal tissues including liver, adipose tissue and the mammary glands18.

We are aware of some limitations in our study. A cause-effect relationship cannot be described between circulating FASN and blood pressure or metabolic variables in pregnant women by the present study design. The exact link between circulating FASN and blood pressure in women with pathological conditions, such as preeclampsia or metabolic disorders cannot be discerned in our healthy study population. Moreover, circulating FASN was taken at a single point in the second trimester. Serial measurements of FASN throughout the pregnancy and in maternal hypertensive or dysglycemic conditions would have added greater value to our present results.

In conclusion, circulating FASN was associated with a more favourable blood pressure and metabolic profile and with lower placent al and birth weight. Circulating FASN and placent al FASN expression were closely related. Future studies will clarify whether circulating FASN of placental origin regulates placental and fetal growth, and (thereby) has a favourable effect on blood pressure and insulin sensitivity in the pregnant mother.

Methods

Study population and ethics. 115 pregnant Caucasian women experiencing normal pregnancies and delivering normal birth weight infants were included in the study. These infants were included in a prenatal cohort of healthy infants. The women were recruited at the Figueres Hospital26 and none of the women experienced complicated pregnancies or parturition. Women with complications, such as preeclampsia, gestational diabetes, fetal malformations, asphyxia and multiple pregnancies were excluded from this prenatal cohort of apparently healthy mothers and babies.

The Dr. Josep Trueta Hospital’s Institutional Review Board approved the protocol. All the women signed an informed written consent. The methods were implemented according to approved guidelines.

Assessments. All patients were subjected to clinical examinations, ultrasonograms and blood tests. Mothers gave information about socio-demographic characteristics, medical data during pregnancy and at delivery. Anthropometric measurements were derived from standard medical records27.

The weight, height, BMI (in Kg/m²) and blood pressure (both systolic and diastolic) of the pregnant women were assessed at each trimester of gestation. An electronic sphygmomanometer (Dinamap Pro 100, GE Healthcare, UK) was used on the right arm and with women in the sitting position to determine the blood pressure.

All infants were born between 37 and 42 weeks and had normal birth weights (from −2.0 to +2.0 DE). Within the first hour after delivery, infants were measured with a measuring board and weighed with a calibrated scale. Birth weight and length were adjusted for gestational age and sex according to regional norms28.

Analytical methods. All serum samples for assessing circulating FASN and metabolic markers were obtained under fasting conditions between 24 and 28 gestation weeks, when glucose tolerance assessment was carried out. All women were subjected to fasting glucose and oral glucose tolerance test (1 h with 50 g glucose).

The hexokinase method was used to analyse serum glucose. To determine HbA1c the ion-exchange HPLC method was used (Bio-Rad Laboratories, S.A. Madrid, Spain). Immuno-chemiluminescence was used to measure serum insulin (IMMULITE 2000, Siemens Healthcare, Madrid, Spain) with a detection limit of 0.4 mU/L and coefficients of variability less than 10%. HOMA-IR was calculated according to the formula: fasting insulin (mU/L) × fasting glucose (mM)/22.5. High-molecular-weight adiponectin was determined with enzyme-linked immunosorbent assay (Linco Research, Missouri, USA) with a limit of detection of 0.5 ng/mL and coefficients of variability less than 4%.

Serum lipids including triglycerides and HDL cholesterol were determined using routine laboratory tests. A quantitative ELISA was used to measure serum FASN concentrations (FASgen Diagnostics, Baltimore, USA) with a limit of detection of 0.3 ng/ml and coefficients of variability less than 12%.

Placental collection. Placental tissue was studied in a representative subgroup (n = 80) of the women studied and who did not differ in clinical or laboratory parameters from the whole group. Placentas were weighed at the moment of delivery. Three fragments of ~1 cm³ of tissue were biopsied on the maternal side for each placenta. The samples were embedded in RNA later (QIAGEN, Madrid, Spain) into cryotubes and stored at −80°C until RNA extraction.

Gene expression. Placental biopsies were each homogenized and total RNA was extracted (RNeasy Mini Kit; QIAGEN, Madrid, Spain) using the standard protocol with DNase. The absorbance at 260 nm was used for assessing RNA concentration (Spectramax Plus 384 Microplate Reader, Molecular Devices, Berkshire, UK). A260/A280 ratio was used to exclude protein contamination.

One microgram of RNA was converted to cDNA following the instructions of the manufacturer (High-Capacity RNA-to-cDNA Kit, Life Technologies SA, Madrid, Spain). To perform real time PCR 25 μl mixtures were prepared containing 2xTaqman Universal Master Mix (12.5 μl) (Applied Biosystems), cDNA (4 ng/μl), and the following Taqman Gene Expression Assays (1.25 μl) (Life Technologies SA, Madrid, Spain): FASN
(Hs00188012), TBP (Hs99999910) and SDHA (Hs00360422). The cycling protocol was 50 °C, 2 min; 95 °C, 10 min; then 40 cycles of 95 °C, 15 s and 60 °C, 1 min (ABI PRISM® 7000 Sequence Detection System; Life Technologies SA, Madrid, Spain). The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression using the Ct values of the housekeeping genes TBP and SDHA.\(^{27}\)

**Statistics.** Results are expressed as mean ± SEM. Statistical analyses were performed using SPSS statistics software (IBM, Madrid, Spain). Logarithmic transformation was used to restore symmetry of non-parametric variables. Pearson correlation test and multiple regression analyses in a stepwise manner were used to analyze the association between quantitative variables. Variables of interest were also analysed by One-Way ANOVA according to tertiles of circulating FASN and $p < 0.05$ was used as the significance level. The study exhibited a power of 80% and a 0.26 Pearson's correlation coefficient to detect a significant association between serum FASN, blood pressure and metabolic parameters, and a 0.30 Pearson's correlation coefficient between serum FASN and placental FASN expression.

**References**

1. Semenkovich, C. F. Regulation of fatty acid synthase (FAS). Prog Lipid Res. 36, 43–53 (1997).
2. Bühler, M. et al. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell. 3, 25–38 (2002).
3. Claycombe, K. J. et al. Insulin increases fatty acid synthase gene transcription in human adipocytes. Am J Physiol. 274, R1253–R1259 (1998).
4. Scott, C. L. Diagnosis, prevention, and intervention for the metabolic syndrome. Am J Cardiol. 92, 351–421 (2003).
5. Berndt, J. et al. Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. Diabetologia. 50, 1472–1480 (2007).
6. Mayas, M. D. et al. Inverse relation between FASN expression in human adipose tissue and the insulin resistance level. Nat Metab (Lond). 3, 1–10 (2017).
7. Roberts, R. et al. Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia. 52, 882–890 (2009).
8. Ranganathan, G. et al. The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. J Lipid Res. 47, 2444–2450 (2006).
9. Oliveras-Ferraros, C., Vazquez-Martín, A., Fernandez-Real, J. M. & Menendez, J. A. AMPK-sensed cellular energy state regulates the release of extracellular Fatty Acid Synthase. Biochem Biophys Res Commun. 378, 488–493 (2009).
10. Kuhajda, F. P. et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci USA. 91, 6379–6383 (1994).
11. Piter, E. S. et al. Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. Cancer Res. 56, 1189–1193 (1996).
12. Valverde, A. M., Benito, M. & Lorenzo, M. The brown adipose cell: a model for understanding the molecular mechanisms of insulin resistance. Acta Physiol Scand. 183, 59–73 (2005).
13. Menendez, J. A. & Lupu, R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 7, 763–777 (2007).
14. Puig, T. et al. Novel Inhibitors of Fatty Acid Synthase with Anticancer Activity. Clin Cancer Res. 15, 7608–7615, doi: 10.1158/1078-0432.CCR-09-0856 (2009).
15. Ueda, S. M. et al. Trophoblastic neoplasms express fatty acid synthase, which may be a therapeutic target via its inhibitor C93. Am J Pathol. 175, 2618–2624 (2009).
16. Marseille-Tremblay, C. et al. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. Mol Reprod Dev. 75, 1054–1062 (2008).
17. Lappas, M. Effect of pre-existing maternal obesity, gestational diabetes and adipokines on the expression of genes involved in lipid metabolism in adipose tissue. Metab. clin. exp. 63, 250–262 (2014).
18. Gonzalez, R. S., Rodriguez-Cruz, M., Maldonado, J. & Saavedra, F. J. Role of maternal tissue in the synthesis of polyunsaturated fatty acids in response to a lipid-deficient diet during pregnancy and lactation in rats. Gene. 549, 7–23 (2014).
19. Mayas, M. D. et al. Decrease in FASN expression in adipose tissue of hypertensive individuals. Am J Hypertens. 22, 1258–1262 (2009).
20. Fernandez-Real, J. M. et al. Extracellular fatty acid synthase: a possible surrogate biomarker of insulin resistance. Diabetes. 59, 1506–1511 (2010).
21. Najjar, S. M. et al. Insulin acutely decreases hepatic fatty acid synthase activity. Cell Metab. 2, 43–53 (2005).
22. Wang, S., Liang, B., Violet, B. & Zou, M. H. Inhibition of the AMP-activated protein kinase-alpha2 accentuates agonist-induced vascular smooth muscle contraction and high blood pressure in mice. Hypertension. 57, 1010–1017 (2011).
23. Young, B. C., Levine, R. J. & Karumanchi, S. A. Pathogenesis of preeclampsia. Annu Rev Pathol. 5, 173–192 (2010).
24. Woollert, L. A. Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation. Am J Clin Nutr. 82, 1155–61 (2005).
25. Wilentz, R. E., Witters, L. A. & Piter, E. S. Lipogenic enzymes fatty acid synthase and acetyl-coenzyme A carboxylase are coexpressed with sterol regulatory element binding protein and Ki-67 in fetal tissues. Pediatr Dev Pathol. 3, 525–31 (2000).
26. Bassols, J. et al. Lower free thyroxin associates with a less favourable metabolic phenotype in healthy pregnant women. J clin endocrinol metab. 96, 3717–3723 (2011).
27. Bassols, J. et al. Placental FTO expression relates to fetal growth. Int J Obes (Lond). 34, 1365–70 (2010).
28. Carrascosa, A. et al. Spanish cross-sectional growth study 2008. Part II. Height, weight and body mass index values from birth to adulthood. An Pediatr (Barc). 68, 552–569 (2008).

**Acknowledgements**

The authors wish to express their sincere gratitude to all the women and their newborns who participated in this study. This study was supported by grants from the Spanish Ministry of Science and Innovation, Carlos III Healthcare Institute (ISC III), Madrid, Spain (MS12/03239 and PI14/01625 to J.B. and PI13/01257 to A.L.-B.), the projects were cofinanced by the FEDER (European Regional Development Fund). F.d.Z. is a Senior Investigator from the Clinical Research Fund of Leuven University Hospital, Belgium. L.I. is a Clinical Investigator from CIBERDEM Center Network for Biomedical Research in Diabetes and Related Metabolic Diseases, from the Carlos III Healthcare Institute, Spain. A.L.-B. is an Investigator from the I3 Fund for Scientific Research (Ministry
of Science and Innovation, Spain). J.B. is an investigator from the Miguel Servet Fund of the Spanish National Institute of Health Carlos III, Spain.

**Author Contributions**

G.C.-B. participated in the acquisition of the data and drafting the paper; A.P.-P. participated in the acquisition and analysis of the data; T.P. participated in the analysis of the data and critical revision of the article; M.V.-R. participated in the acquisition of the data and critical revision of the article; M.B. participated in the acquisition of the data and critical revision of the article; E.M. participated in the acquisition of the data and critical revision of the article; F.d.Z. participated in the analysis of the data and critical revision of the article; L.I. participated in the analysis of the data and critical revision of the article; A.L.-B. participated in the conception, analysis of the data and critical revision of the article. J.B. participated in the conception, analysis of the data and critical revision of the article.

**Additional Information**

**Supplementary information** accompanies this paper at http://www.nature.com/srep

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Carreras-Badosa, G. et al. Circulating Fatty Acid Synthase in pregnant women: Relationship to blood pressure, maternal metabolism and newborn parameters. *Sci. Rep.* 6, 24167; doi: 10.1038/srep24167 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/