Free Leptin Index is Elevated in Preeclamptic but not Healthy Women throughout Gestation. A Prospective Cohort Study

María Carolina Páez - Leal
Department of Public Health, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

María Fernanda Garces
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

María Alejandra Cano – Bermudez
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Luis Miguel Maldonado - Acosta
Division of Endocrinology - Department of Internal Medicine. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Jhon Jairo Peralta - Franco
Division of Endocrinology - Department of Internal Medicine. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Álvaro Javier Burgos - Cardenas
Department of Internal Medicine. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Edith Ángel - Müller
Department of Obstetrics and Gynecology. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Arturo José Parada – Baños
Department of Obstetrics and Gynecology. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Javier Eslava – Schmalbach
Department of Surgery, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Haiver Antonio Rodríguez - Navarro
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Juliet Daniela Buell - Acosta
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Justo Castaño
Maimonides Institute of Biomedical Research of Cordoba (IMIBIC); Department of Cell Biology, Physiology and Immunology, University of Córdoba; Reina Sofia University Hospital

Rubén Nogueiras
Department of Physiology (CIMUS), School of Medicine - Instituto de Investigaciones Sanitarias (IDIS), Universidad de Santiago de Compostela, Santiago de Compostela

Carlos Dieguez
Department of Physiology (CIMUS), School of Medicine - Instituto de Investigaciones Sanitarias (IDIS), Universidad de Santiago de Compostela, Santiago de Compostela

Elizabeth Sanchez
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Ariel Iván Ruiz – Parra
Department of Obstetrics and Gynecology. School of Medicine Universidad Nacional de Colombia, Bogota Colombia

Jorge Eduardo Caminos (jecaminosp@unal.edu.co)
Department of Physiology, School of Medicine Universidad Nacional de Colombia, Bogota Colombia

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Abstract

The ratio leptin/soluble leptin receptor (sOB-r), free leptin index (FLI), is used as a marker of leptin sensitivity/resistance in different pathologies. The aim of this study was to evaluate FLI in healthy non-pregnant, healthy pregnant and mild preeclamptic women during pregnancy. We conducted a nested case-control study within a longitudinal observational prospective cohort study. Serum leptin (p=0.0001) and sOB-r (p=0.0000) levels rose significantly throughout pregnancy in healthy pregnant and preeclamptic women [leptin (p=0.0000); sOB-r (p=0.0380)]. Serum leptin levels were significantly higher in preeclamptic compared to healthy pregnant women at 2nd (p=0.0245) and 3rd trimesters of pregnancy (p=0.0016). Additionally, serum sOB-r levels were significantly lower in preeclamptic women during the 2nd (p=0.0236) and 3rd trimester (p=0.0024) of pregnancy compared to healthy pregnant women. Moreover, we found that FLI did not vary significantly during any of the three periods studied in healthy pregnant women (p=0.7640), whereas, increased throughout preeclamptic pregnancy (p=0.0037). Indeed, FLI was significantly higher at 2nd (p=0.0053) and 3rd (p=0.0003) trimesters of pregnancy in preeclamptic compared to healthy pregnant women. Additionally, FLI was significantly higher during luteal phase compared to the follicular phase (p=0.0039). These results demonstrate that FLI increases significantly in preeclamptic pregnant women towards the end of pregnancy.

1. Introduction

Leptin is an adipokine predominantly synthetized and secreted by adipocytes, but it is also produced at lower levels in other tissues, including the placenta, therefore playing pleiotropic roles in the control of energy metabolism, reproductive function, immunity, and bone metabolism, where it exerts these actions by binding to specific cell surface receptor1. Leptin receptors belong to the class I cytokine receptor family, and six splice variants with identical ligand-binding domains and alternative or truncate cytoplasmic domains have been described (LepRa, LepRb, LepRc, LepRd, LepRe and LepRf)1,2. Furthermore, the soluble leptin receptor (Ob-Re or sOB-r) represents the major leptin binding protein in blood, and plays an important role in leptin signaling3-9.

Previous studies have demonstrated that circulating leptin levels increase significantly during normal pregnancy, reaching a nadir in the third trimester and returning to preconception values in the postpartum period15. Additionally, some studies have shown that chronically higher plasma leptin levels are associated with obesity, metabolic syndrome and gestational diabetes mellitus 7,11-16. Leptin also affects blood pressure and contribute to hypertension through sympathetic nervous system activation on both vasculature and the kidney 17,18. During normal pregnancy basal sympathetic nerve activity increases 19 and deregulation of leptin levels has been correlated with the pathogenesis of preeclampsia 10,20-23. For instance, leptin expression is increased in preeclamptic placentas and leptin concentrations are higher in preeclamptic women compared with normotensive pregnant women24-26.

The rate between circulating leptin and sOB-r, known as free leptin index (FLI = leptin/sOB-r), has been widely used to study leptin sensitivity/resistance 26,27. FLI is significantly increased in obese patients, due to higher circulating levels of leptin and lower levels of sOB-r compared to healthy normal weight control or obese subjects undergoing a weight reduction diet 28-30. Thus, high FLI values have been associated with progressive and chronic diseases such as obesity, type 2 diabetes (T2D), reproductive diseases and nonalcoholic fatty liver disease (NAFLD) 13,31-34. In this way, FLI has been associated with a number of pathological pregnancy states, including gestational diabetes mellitus (GDM) and preeclampsia 7,13,35.

To date, some cross-sectional studies have reported that FLI is increased in preeclamptic compared with healthy pregnant women35-37. However, FLI has not been evaluated longitudinally in mild preeclamptic pregnant women. Therefore, the current study aims to investigate this index throughout pregnancy in healthy and mild preeclamptic pregnant women in a case-control study nested within a longitudinal prospective cohort.

2. Material And Methods

2.1. Ethical consideration

The study protocol was approved by the Institutional Ethics Committee of the School of Medicine of the Universidad Nacional de Colombia (Ref. No. 011–165–18; June 2018). The study was conducted according to the revised Declaration of Helsinki and all participants were informed about the study and those who agreed to participate read and signed a consent form to participate in the study. This study was conducted in the Gynecology and Obstetrics Department of the School of Medicine – Universidad Nacional de Colombia and The Engativa Hospital – Bogotá, between May 2012 and November 2015.

2.2. Study Design and Participants

A nested case-control study within a longitudinal observational prospective cohort study (n = 465) was carried out to compare maternal FLI in healthy and preeclamptic pregnant women, across the three trimesters of pregnancy and three months postpartum. Study participants were recruited among pregnant women attending the obstetrics and gynecology health promotion and disease prevention program at the Engativa Hospital - Bogota. Pregnant women were recruited at 1st trimester (11–13 weeks) of pregnancy and followed until delivery and up to three months postpartum. Gestational age was calculated according to the last menstrual period or ultrasound examination in the first trimester. Study subjects included healthy pregnant women (n = 43) and woman diagnosed with mild preeclampsia (n = 20) randomly selected from the original cohort (n = 465). Furthermore, twenty healthy non-pregnant women with regular menstrual cycles were included in the study during the follicular and luteal phases of the menstrual cycle.

Demographic, medical and reproductive clinical history was obtained by verbal interview. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined and mean arterial pressure (MAP) was calculated. Additionally, follow-up assessments of mothers as part of routine antenatal care visit included anthropometric, biochemical and hormonal determinations. Women with mild preeclampsia and late-onset preeclampsia, were delivered at ≥ 34 weeks’ gestation and diagnosed and classified according to the ACOG guidelines and elsewhere [Systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg on two occasions taken separated by a 4 to 6 hour period, and proteinuria ≥ 300 mg/24 hour or ≥ 2 + dipstick]38,39. Mild to moderate
preeclampsia with clinical manifestation occurring after 34 weeks were detected through routine prenatal screening and diagnosed with systolic blood pressure between 140 to 159 mmHg or diastolic blood pressure measures between 90 to 109 mmHg, non-elevated liver enzymes, absence of renal insufficiency, pulmonary edema, cyanosis, new-onset headaches or visual disturbances, and/or right upper quadrant or epigastric pain. Data about maternal health complications, time and type of delivery, and neonatal characteristics at birth were retrieved from medical records.

Additionally, for the present study we excluded women with multiple gestations or the development of any complications during pregnancy, including pre-pregnancy hypertension, gestational diabetes mellitus, autoimmune and metabolic disorder, thyroid disease, liver and renal disease, acute and chronic infections, and diseases of the hematopoietic system, as well as women who were taking medications that affected metabolism.

### 2.3. Biochemical analysis

All biochemical and hormonal laboratory measurements were performed in the morning hours (07:00–08:00 hours) following an overnight fast (10:00–12:00 hours). Blood samples from the pregnant and non-pregnant women were collected at each visit into BD Vacutainer® tubes from veins in the antecubital area. Serum glucose, total serum cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) levels were measured by enzymatic methods (Labkit Kits, Spain). Additionally, serum insulin levels were measured by electrochemiluminescence immunoassay using Roche Modular detection system (Roche, Basel, Switzerland) and serum C-reactive protein (CRP) were measured by colorimetric enzyme-linked immunosorbent assay (ELISA). The Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR) was determinate according to the following formula: HOMA-IR = [fasting glucose (mmol/L) X fasting insulin (mU/mL)] /22.52. Serum progesterone levels were measured in healthy non-pregnant women by immunoassay (Roche Elecsys 1010 Immunoanalyzer Boulder, Colorado, USA) in serum samples obtained during the follicular and luteal phases of the menstrual cycle.

Human serum leptin (Invitrogen, KAC2281) and sOB-r (DOBR00, R&D Systems) were determined in duplicate using the commercially available ELISA kits and assays were performed as described by the manufacturer. For serum human leptin, the analytical sensitivity was < 3.5 pg/mL, the assay range 15.6–1000 pg/mL and the intra- and inter-assay coefficient of variation (CV) were 4.6% and 3.6%, respectively. Additionally, for human serum sOB-r ELISA, the analytical sensitivity was < 0.128 ng/mL, the assay range 0.3–20 ng/mL and the intra- and inter-assay coefficient of variation (CV) were 5.5% and 5.5%, respectively. The ratio between circulating leptin to sOB-r levels (leptin/sOB-r) (FLI) was determined as described elsewhere.

### 2.4. Statistical analyses

Variables were expressed as means ± standard deviation (SD) or median and interquartile range (IQR) if they were parametric or not parametric. Parametric variables were compared using the Student t-test and one-way analysis of variance (ANOVA). We used the Bonferroni test, if the results were statistically significant. Non-parametric variables were evaluated with the Mann-Whitney test. For the longitudinal study, where the data were normally distributed, a repeated measures ANOVA was used with a Bonferroni post hoc test. For non-parametric longitudinal data, a Kruskal Wallis test was used with a Dunn's post hoc test. A p-value < 0.05 was considered statistically significant in all analyses, with a 95% confidence interval (CI). We used STATA 15-IC® version for statistical analyses.

### 3. Results

#### 3.1. Serum leptin levels are elevated throughout gestation and are higher in preeclamptic women

Demographic characteristics, clinical data and biochemical parameters of the study group are present in Tables 1 and 2. Serum leptin concentrations were significantly increased in the luteal phase compared to follicular phase of the menstrual cycle in the non-pregnant women (p = 0.0000) (Fig. 1 and Supplementary Table 1). In healthy pregnant women, circulating leptin levels increased significantly throughout gestation (p = 0.0000). This increment was statistically different between first and second trimester of pregnancy, whereas no significant differences were found between second and third trimester (Fig. 1 and Supplementary Table 1). After delivery, leptin levels markedly dropped (Fig. 1 and Supplementary Table 1).
Table 1
Demographic, clinical and biochemical parameters in the studied population of healthy pregnant women and healthy non-pregnant women.

| Variables                          | Non-pregnant (n=19) | Healthy Pregnant Women (n = 43) | Post-partum (n = 18) |
|------------------------------------|---------------------|---------------------------------|---------------------|
|                                    |                     | 1st trimester                  | 2nd trimester       | 3rd trimester |                  |
| Age (years)                        | 22,26 (± 3,75)      | 25,06 (± 6,65)                 | -                   | -            | -                |
|                                    | 19 - 25             | 19 - 31                         | -                   | -            | -                |
| Gestational age (weeks)            | -                   | 12,12 (± 0,63)                 | 24,45 (± 0,69)      | 34,75 (± 0,95) | -                |
|                                    |                     | 11,5 - 12,5                    | 24,1 - 24,6         | 34,2 - 35,4  | -                |
| BMI (kg/m^2)                       | 21,26 (± 1,75)      | 22,73 (± 2,28)                 | 24,63 (± 2,42)      | 26,49 (± 2,57) | 23,12 (± 2,47)  |
|                                    | 19,9 - 22,9         | 20,8 - 23,8                    | 22,7 - 25,9         | 24,4 - 27,9  | 21 - 24,4        |
| PAS (mmHg)                          | 106,94 (± 9,74)     | 93,37 (± 7,53)                 | 90,88 (± 9,05)      | 96,09 (± 8,48) | 105,66 (± 24,22)|
|                                    | 99 - 115            | 90 - 100                        | 82 - 100            | 90 - 102     | 108 - 116        |
| PAD (mmHg)                          | 69 (± 5,93)         | 60,58 (± 6,10)                 | 59,48 (± 6,40)      | 62,09 (± 8,01)| 68,06 (± 4,87)  |
|                                    | 65 - 75             | 58 - 62                        | 58 - 60             | 58 - 64      | 65 - 70          |
| PAM (mmHg)                          | 81,64 (± 6,34)      | 71,51 (± 5,83)                 | 69,95 (± 6,04)      | 73,42 (± 7,54)| 80,59 (± 7,74)  |
|                                    | 76,67 - 87,3        | 69,33 - 74                     | 66,67 - 73,33       | 69,3 - 78    | 78,3 - 84        |
| Blood glucose (mg/dL)              | 82,2 (± 7,46)       | 78,87 (± 5,91)                 | 74,54 (± 5,33)      | 74,23 (± 5,73)| 81,44 (± 5,92)  |
|                                    | 78 - 86             | 74 - 83                        | 69 - 79             | 71 - 77      | 77 - 84          |
| Insulin (µU/mL)                    | 9,14 (± 5,67)       | 9,61 (± 4,26)                  | 11,34 (±4,26)       | 11,95 (± 5,21)| 6,57 (±3,79)    |
|                                    | 4,5 - 14,1          | 5,9 - 11,7                     | 8,5 - 14,3          | 7,7 - 16,7   | 3,9 - 9          |
| HOMA Index                         | 1,69 (±1,25)        | 1,88 (±0,89)                   | 2,10 (±0,86)        | 2,21 (±1,02) | 1,33 (±0,79)    |
|                                    | 0,84 - 2,35         | 1,17 - 2,22                    | 1,52 - 2,64         | 1,41 - 3,05  | 0,73 - 1,87     |
| Total cholesterol (mg/dL)          | 157,31 (± 27,26)    | 166,69 (± 31,61)               | 221,41 (± 39,25)    | 251,44 (± 50,56)| 158,66 (± 28,3) |
|                                    | 129 - 178           | 145 - 190                      | 190 - 255           | 219 - 287    | 140 - 181        |
| HDL (mg/dL)                        | 47,84 (± 8,69)      | 58,60 (± 9,92)                 | 70,32 (± 12,35)     | 66,48 (± 11,27)| 45,88 (± 10,26) |
|                                    | 43 - 52             | 51 - 65                        | 62 - 78             | 64 - 74      | 44 - 53          |
| LDL (mg/dL)                        | 109,52 (± 27,24)    | 122,48 (± 34,43)               | 146,18 (± 47,06)    | 162 (± 44,77) | 94,66 (± 29,69) |
|                                    | 90 - 127            | 94 - 148                       | 109 - 177           | 134 - 190    | 74 - 113         |
|                             | 15.26 (± 4.70) | 22.44 (± 8.11) | 37.39 (± 12.41) | 49.69 (± 15.14) | 18.33 (± 10.71) |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 12 - 19        | 16 - 27        | 28 - 44        | 41 - 58        | 12 - 23        |

| Triglycerides (mg/dL)       | 76.05 (± 23.36) | 112.16 (± 40.26) | 187.04 (± 82.02) | 248.44 (± 75.75) | 92 (± 53.67) |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 59 - 63        | 81 - 133       | 140 - 221      | 205 - 291      | 59 - 116       |

| C-Reactive protein (mg/dL)  | 1.55 (± 1.46)  | 5.41 (± 2.69)  | 4.82 (± 2.42)  | 5.38 (± 3.30)  | 3.51 (± 3.93)  |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 0.56 - 1.64    | 3.7 - 7.44     | 3.07 - 6.55    | 2.59 - 8.37    | 1.3 - 4.33     |

| Progesterone (mg/dL)        | 0.50 (± 0.23)  | -              | -              | -              | -              |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 0.33 - 0.67    | -              | -              | -              | -              |

| Leptin (ng/mL)              | 16.52 (± 6.63) | 22.83 (± 9.34) | 34.38 (± 18.22) | 38.24 (± 19.51) | 16.03 (± 4.89) |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 13.78 - 17.32  | 16.38 - 30.07  | 21.98 - 46.67  | 21.68 - 52.65  | 11.6 - 20.17   |

| Leptin (ng/mL)              | 22.94 (± 6.37) | -              | -              | -              | -              |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 19.22 - 27.31  | -              | -              | -              | -              |

| Leptin (ng/mL)              | 19.94 - 22.73  | 28.13 - 36.61  | 37.94 - 49.16  | 35.70 - 53.34  | 23.55 - 29.96  |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 21.60 (± 2.61) | -              | -              | -              | -              |

| Leptin (ng/mL)              | 18.89 - 23.52  | -              | -              | -              | -              |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 10.85 (± 5.56) | -              | -              | -              | -              |

| FLI (ng/mL)                 | 7.87 (± 2.60)  | 7.80 (± 4.85)  | 8.62 (± 6.38)  | 9.35 (± 5.87)  | 6 (± 1.57)     |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
|                             | 6.21 - 8.46    | 4.64 - 10.26   | 4.83 - 10.79   | 4.16 - 11.92   | 4.85 - 7.67    |

Demographic, clinical and biochemical parameters in the studied population of healthy pregnant and healthy non-pregnant women. Normal distribution was indicated as mean (SD) and non-normal parameters were indicated as median (IQR). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HOMA-IR: homeostasis model assessment-estimated insulin resistance; FLI: free leptin index. A p-value < 0.05 was considered statistically significant.
Table 2
Demographic, clinical and biochemical parameters of the studied population of preeclamptic women.

| Variables                      | Preeclamptic women (n= 20) |
|--------------------------------|-----------------------------|
|                                | 1st trimester | 2nd trimester | 3rd trimester |
| Age (years)                    | 22.35 (± 6.58) | - | - |
| range                          | 18 - 26.5 | - | - |
| Gestational age (weeks)        | 12.17 (± 0.69) | 24.45 (± 0.57) | 35 (± 0.86) |
| range                          | 11.5 - 12.6 | 24.05 - 24.5 | 34.2 - 35.55 |
| BMI, kg/m²                     | 23.97 (± 2.81) | 26.36 (± 2.82) | 29.37 (± 2.73) |
| range                          | 21.8 - 25.25 | 24.35 - 28.05 | 27.7 - 30.55 |
| PAS (mmHg)                     | 104 (± 7.57) | 104.2 (± 8.63) | 108.55 (± 12.46) |
| range                          | 99 - 110 | 100 - 110 | 100 - 115 |
| PAD (mmHg)                     | 65.7 (± 7.6) | 65.4 (± 7.45) | 65.25 (± 6.64) |
| range                          | 60 - 70 | 60 - 70 | 60 - 70 |
| PAM (mmHg)                     | 78.46 (± 7.09) | 78.33 (± 6.93) | 79.68 (± 9.22) |
| range                          | 73 - 82.65 | 74 - 83.35 | 74.5 - 81.65 |
| Blood glucose (mg/dL)          | 80.48 (± 6.77) | 77.05 (± 7.72) | 74.68 (± 9.25) |
| range                          | 75.3 - 84 | 70 - 83 | 69.5 - 78 |
| Insulin (µUI/mL)               | 17.25 (± 23.75) | 15.28 (± 4.46) | 15.28 (± 6.57) |
| range                          | 9.75 - 13.85 | 11.2 - 18.15 | 11.45 - 18.55 |
| HOMA Index                     | 3.68 (± 5.9) | 2.9 (± 0.86) | 2.85 (± 1.36) |
| range                          | 1.84 - 2.86 | 2.22 - 3.61 | 2.13 - 3.52 |
| Total Cholesterol (mg/dL)      | 171.05 (± 32.95) | 220.65 (± 44.41) | 235.75 (± 48.47) |
| range                          | 159.5 - 191.5 | 189 - 244 | 207.5 - 258.5 |
| HDL (mg/dL)                    | 52.25 (± 12.11) | 63.65 (± 14.75) | 57.65 (± 17.59) |
| range                          | 44 - 58.5 | 51.5 - 74 | 51 - 63.5 |
| LDL (mg/dL)                    | 122.65 (± 38.89) | 152.85 (± 57.97) | 157.9 (± 68.28) |
| range                          | 91.5 - 143 | 113 - 174.5 | 110 - 198.5 |
| VLDL (mg/dL)                   | 23.05 (± 9.47) | 35.9 (± 14.85) | 49.85 (± 19.32) |
| range                          | 16 - 27 | 26.5 - 42 | 33.5 - 64 |
| Triglycerides (mg/dL)          | 115.15 (± 47.28) | 179.45 (± 73.87) | 258.9 (± 85.37) |
| range                          | 78 - 134.5 | 133 - 208.5 | 187 - 321 |
| Creactive protein (CRP)        | 115.15 (± 47.28) | 7.42 (± 2.99) | 7.09 (± 3.46) |
| range                          | 78 - 134.5 | 5.27 - 10 | 5.24 - 8.73 |
| Leptin (ng/mL)                 | 24.91 (± 9.91) | 47.11 (± 25.11) | 63.01 (± 30.92) |
| range                          | 16.97 - 31.78 | 29.25 - 61.15 | 32.2 - 83.31 |
| sOB-r (ng/mL)                  | 32.09 (± 6.97) | 37.54 (± 6.33) | 36.97 (± 7.66) |
| range                          | 25.7 - 37.85 | 32.88 - 43.8 | 30.97 - 42.9 |
| FLI                            | 8.69 (± 4.969) | 13.54 (± 8.78) | 18.06 (± 10.35) |
| range                          | 4.62 - 12.38 | 7.36 - 19.54 | 7.73 - 25.69 |

Demographic, clinical and biochemical parameters of preeclamptic women. Normal distribution was indicated as mean (SD) and non-normal parameters were indicated as median (IQR). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; HOMA-IR: homeostasis model assessment-estimated insulin resistance; FLI: free leptin index. A p-value < 0.05 was considered statistically significant.

In preeclamptic women, leptin levels are also significantly elevated throughout gestation (p = 0.0000) (Fig. 1 and table 2). When second (p = 0.0245) and third (p = 0.0016) trimester of preeclamptic women are compared to second and third trimester of healthy pregnant women, a statistically significant difference is
found in serum leptin levels (Fig. 1 and Supplementary Table 2).

3.2. Serum sOB-r levels are increased during pregnancy in healthy and preeclamptic women

On the other hand, serum sOB-r concentrations were not statistically different between follicular and luteal phases of the menstrual cycle in the non-pregnant women (p = 0.3969) (Fig. 2 and Supplementary Table 3). In healthy pregnant women, a significant increase was observed in serum sOB-r concentrations from first to second and third trimesters of pregnancy (p = 0.0000) and there were no statistically significant differences between second and third trimesters (p = 0.3999) (Fig. 2 and Supplementary Table 3). Serum sOB-r levels were significantly decreased after delivery (Fig. 2 and Supplementary Table 3).

Circulating sOB-r was also significantly elevated throughout gestation in preeclamptic women (p = 0.0174) (Fig. 2), where serum sOB-r levels during the second (p = 0.0236) and third trimester (p = 0.0024) were higher than in the first trimester. In addition, serum sOB-r levels were similar between healthy pregnancy and preeclamptic women (Fig. 2 and Supplementary Table 4).

3.3. Free leptin index is increased throughout pregnancy in preeclamptic, but not in healthy women

The FLI was lower in the follicular than in luteal phase of menstrual cycle of non-pregnant women (Fig. 3 and Supplementary Table 5) (p = 0.0039). FLI was not significantly different at any trimester of pregnancy in healthy pregnant women (p = 0.7640) (Fig. 3). Conversely, FLI increased significantly from the first to third trimesters of pregnancy in preeclamptic pregnant women (Fig. 3) (p = 0.0037). The FLI was significantly elevated during the second (p = 0.0053) and third trimester (p = 0.0003) of gestation in preeclamptic women when compared to healthy pregnant women (Fig. 3 and Supplementary table 6).

4. Discussion

The present study demonstrates, for the first time that, at variance to pregnant healthy women, FLI is significantly elevated during pregnancy in mild preeclamptic women. The FLI in the second and third trimesters of gestation in mild preeclamptic women was higher than in healthy pregnant women at the same periods, in a nested case-control study within a prospective cohort study. This significant increase occurs particularly in the second and third trimesters of pregnancy as result of the notable increased circulating levels of leptin and the moderated elevation in serum levels of sOB-r in preeclamptic women. These results are consistent with the previous findings reported by Andersson-Hall et al. in healthy pregnant women in a longitudinal prospective cohort study[^13]. Additionally, it is important to note that FLI was significantly higher in the luteal versus follicular phase of the menstrual cycle, in response to higher serum leptin concentrations in the luteal phase.

Preeclampsia is a multisystem hypertensive disorder of pregnancy and one of the major causes of maternal and fetal morbidity and mortality worldwide[^42]. In addition, it has been associated with endothelial dysfunction, coagulopathies, imbalance between angiogenic and anti-angiogenic factors, acute kidney injury, edema, and development of cardiovascular diseases, systemic inflammatory response and oxidative stress[^43]. Furthermore, preeclampsia has been associated with a dysregulated secretion profile of maternal and placental circulating factor, including growth factors, hormones and some adipokines such as leptin[^44].

Leptin is an adipokine produced and secreted primarily by white adipose tissue, which crosses the blood–brain barrier through a saturable transport system to reach the hypothalamus and activate the sympathetic nervous system that can lead to hypertension[^45–47]. Previous studies have shown that high-circulating leptin levels are present in animals and humans with hypertension[^45–49]. Here, we found that circulating levels of leptin are significantly elevated during the second and third trimesters of gestation in both healthy and preeclamptic women. However, this increase is more remarkable in preeclamptic pregnant women, suggesting that high leptin levels could contribute to the pathophysiology and underlying mechanisms of hypertensive disorders during pregnancy. Consequently, this index might be helpful as an early predictive biomarker for this hypertensive disorder during pregnancy. Moreover, our study reveals that elevated serum leptin levels and a slightly increased concentration of sOB-r exist in mild preeclamptic women when compared with normotensive pregnant women in the second and third trimesters of pregnancy. These results are consistent with findings reported in patients with primary hypertension, and support the hypothesis that there is a strong relationship between hypertension, leptin and its sOB-r[^50]. Of note, our present results compare favorably and extend those of a previous report showing that hyperleptinemia may precede and contribute to the development of hypertension, rather than being a major cause of it, inasmuch as, in the present study, hyperleptinemia occurs in preeclamptic pregnant women from the second trimester of gestation[^33]. Also, FLI was more strongly related with adverse clinical outcomes and associated with masked hypertension than leptin or sOB-r alone, suggesting that leptin and its receptor acting conjointly may be involved in the hypertensive disorders of pregnancy.

5. Conclusions

This longitudinal study indicates that circulating levels of leptin and sOB-r could be considered an independent risk factor for hypertensive disorders that occur in pregnant women. We suggest that higher leptin concentration might play and important role in the pathophysiology of preeclampsia, and FLI could be useful in predicting the onset of hypertensive disorders during pregnancy.

Declarations

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Author Contributions:
All authors have contributed to the intellectual content of this manuscript: a) A.I.R.P., E.A.M., J.E.C., J.P.C., C.D., R.N., JES, L.M.M.A. and M.C.P.L. conceived and designed the experiments, b) M.F.G., H.A.R.N., M.A.C.B. and J.D.B.A. performed the experiments, c) J.E.C., J.E.C., A.I.R.P., A.J.P.B., J.J.P.F. and A.J.B.C. analyzed the data and d) A.I.R.P., E.A.M., J.E.C., J.P.C., C.D., R.N., JES, L.M.M.A., A.J.P.B., J.J.P.F. and M.C.P.L. prepared the original draft and edited manuscript.

References
1. Wauman, J., Zabeau, L. & Tavernier, J. The Leptin Receptor Complex: Heavier Than Expected? *Front. Endocrinol.* **8**, (2017).
2. Gruzdeva, O., Borodkina, D., Uchasova, E., Dyleva, Y. & Barbarash, O. Leptin resistance: underlying mechanisms and diagnosis. *Diabetes Metab. Syndr. Obes. Targets Ther.* **Volume 12**, 191–198 (2019).
3. Lammert, A., Kiess, W., Bottner, A., Glasow, A. & Kratzsch, J. Soluble Leptin Receptor Represents the Main Leptin Binding Activity in Human Blood. *Biochem. Biophys. Res. Commun.* **283**, 982–988 (2001).
4. Huang, L., Wang, Z. & Li, C. Modulation of Circulating Leptin Levels by Its Soluble Receptor. *J. Biol. Chem.* **276**, 6343–6349 (2001).
5. Schaab, M. & Kratzsch, J. The soluble leptin receptor. *Best Pract. Res. Clin. Endocrinol. Metab.* **29**, 661–670 (2015).
6. Yang, G., Ge, H., Boucher, A., Yu, X. & Li, C. Modulation of Direct Leptin Signaling by Soluble Leptin Receptor. *Mol. Endocrinol.* **18**, 1354–1362 (2004).
7. Hinkle, S. N. *et al.* Maternal adipokines longitudinally measured across pregnancy and their associations with neonatal size, length, and adiposity. *Int. J. Obes.* **43**, 1422–1434 (2019).
8. Zastrov, O. *et al.* The soluble leptin receptor is crucial for leptin action: evidence from clinical and experimental data. *Int. J. Obes.* **27**, 1472–1478 (2003).
9. Zhang, J. & Scarpace, P. J. The soluble leptin receptor neutralizes leptin-mediated STAT3 signalling and anorexic responses in vivo: Soluble receptor neutralizes leptin action in vivo. *Br. J. Pharmacol.* **158**, 475–482 (2009).
10. Pérez-Pérez, A. *et al.* Leptin action in normal and pathological pregnancies. *J. Cell. Mol. Med.* (2017) doi:10.1111/jcmm.13369.
11. Schubring, C. *et al.* Longitudinal Analysis of Maternal Serum Leptin Levels during Pregnancy, at Birth and Up To Six Weeks after Birth: Relation to Body Mass Index, Skinfolds, Sex Steroids and Umbilical Cord Blood Leptin Levels. *Horm. Res. Paediatr.* **50**, 276–283 (1998).
12. Briffa, J. F., McAinch, A. J., Romano, T., Wlodek, M. E. & Hryciw, D. H. Leptin in pregnancy and development: a contributor to adulthood disease? *Am. J. Physiol.-Endocrinol. Metab.* **308**, E335–E350 (2015).
13. Andersson-Hall, U. *et al.* Longitudinal changes in adipokines and free leptin index during and after pregnancy in women with obesity. *Int. J. Obes.* **44**, 675–683 (2020).
14. Baratto, I., Daher, S., Lobo, T. F., Araujo Júnior, E. & Guazzelli, C. A. F. Adiponectin and leptin serum levels in normal adolescent pregnancies. *J. Matern. Fetal Neonatal Med.* 1–6 (2019) doi:10.1080/14767058.2019.1651836.
15. Highman, T. J., Friedman, J. E., Huston, L. P., Wong, W. W. & Catalano, P. M. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. *Am. J. Obstet. Gynecol.* **178**, 1010–1015 (1998).
16. Bartha, J. L., Romero-Carmona, R., Escobar-Llompart, M. & Comino-Delgado, R. The relationships between leptin and inflammatory cytokines in women with pre-eclampsia. *Br. J. Obstet. Gynaecol.* **108**, 1272–1276 (2001).
17. Eikelis, N., Schlaich, M., Aggarwal, A., Kaye, D. & Esler, M. Interactions Between Leptin and the Human Sympathetic Nervous System. *Hypertension* **41**, 1072–1079 (2003).
18. Khatriya, S. *et al.* Obesity Hypertension: The Regulatory Role of Leptin. *Int. J. Hypertens.* **2011**, 1–8 (2011).
19. Reyes, L. M., Usselman, C. W., Davenport, M. H. & Steinback, C. D. Sympathetic Nervous System Regulation in Human Normotensive and Hypertensive Pregnancies. *Hypertension* **71**, 793–803 (2018).
20. Fischer, T. *et al.* Pregnancy-induced sympathetic overactivity: a precursor of pre eclampsia*. *Eur. J. Clin. Invest.* **34**, 443–448 (2004).
21. Schobel, H. P., Fischer, T., Heuszer, K., Geiger, H. & Schmieder, R. E. Preeclampsia — A State of Sympathetic Overactivity. *N. Engl. J. Med.* **335**, 1480–1485 (1996).
22. Greenwood, J. P., Scott, E. M., Stoker, J. B., Walker, J. J. & Mary, D. A. S. G. Sympathetic Neural Mechanisms in Normal and Hypertensive Pregnancy in Humans. *Circulation* **104**, 2200–2204 (2001).
23. Jarvis, S. S. et al. Sympathetic activation during early pregnancy in humans: Muscle sympathetic nerve activity in early pregnancy. J. Physiol. 590, 3535–3543 (2012).

24. Song, Y. et al. Serum levels of leptin, adiponectin and resistin in relation to clinical characteristics in normal pregnancy and preeclampsia. Clin. Chim. Acta 458, 133–137 (2016).

25. Kalinderis, M. et al. Serum levels of leptin and IP-10 in preeclampsia compared to controls. Arch. Gynecol. Obstet. 292, 343–347 (2015).

26. Yannakoulia, M. et al. Body Fat Mass and Macronutrient Intake in Relation to Circulating Soluble Leptin Receptor, Free Leptin Index, Adiponectin, and Resistin Concentrations in Healthy Humans. J. Clin. Endocrinol. Metab. 88, 1730–1736 (2003).

27. Owecki, M., Nikisch, E., Miczke, A., Pupek-Musialik, D. & Sowiński, J. Leptin, Soluble Leptin Receptors, Free Leptin Index, and Their Relationship with Insulin Resistance and BMI: High Normal BMI is the Threshold for Serum Leptin Increase in Humans. Horm. Metab. Res. 42, 585–589 (2010).

28. Herrick, J. E., Panza, G. S. & Gollie, J. M. Leptin, Leptin Soluble Receptor, and the Free Leptin Index following a Diet and Physical Activity Lifestyle Intervention in Obese Males and Females. J. Obes. 2016, 1–5 (2016).

29. Hestiantoro, A. et al. Dysregulation of Kisspeptin and Leptin, as Anorexigenic Agents, Plays Role in the Development of Obesity in Postmenopausal Women. Int. J. Endocrinol. 2019, 1–8 (2019).

30. van Dielen, F. M. H., van ’t Veer, C., Buurman, W. A. & Greve, J. W. M. Leptin and Soluble Leptin Receptor Levels in Obese and Weight-Losing Individuals. J. Clin. Endocrinol. Metab. 87, 1708–1716 (2002).

31. Pivonello, R. et al. Metabolic Disorders and Male Hypogonadotropic Hypogonadism. Front. Endocrinol. 10, 345 (2019).

32. Cernea, S., Roiban, A. L., Both, E. & Huţanu, A. Serum leptin and leptin resistance correlations with NAFLD in patients with type 2 diabetes. Diabetes Metab. Res. Rev. 34, e3050 (2018).

33. Thomopoulos, C. et al. Free Leptin Is Associated With Masked Hypertension in Nonobese Subjects: A Cross-Sectional Study. Hypertension 53, 965–972 (2009).

34. Allison, M. A. et al. Higher leptin is associated with hypertension: the Multi-Ethnic Study of Atherosclerosis. J. Hum. Hypertens. 27, 617–622 (2013).

35. De Villiers, C. P. et al. Placental protein-13 (PP13) in combination with PAPP-A and free leptin index (fLI) in first trimester maternal serum screening for severe and early preeclampsia. Clin. Chem. Lab. Med. CCLM 56, (2017).

36. Krizova, J. et al. Soluble Leptin Receptor and Leptin Levels in Pregnant Women Before and After Delivery. Endocr. Res. 30, 379–385 (2004).

37. Hedley, P. L. et al. Free leptin index and PAPP-A: a first trimester maternal serum screening test for pre-eclampsia: LEPTIN AND PAPP-A SCREENING FOR PRE-ECLAMPSIA. Prenat. Diagn. 30, 103–109 (2010).

38. Poon, L. C. et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Int. J. Gynecol. Obstet. 145, 1–33 (2019).

39. Sisti, G. & Colombi, I. New blood pressure cut off for preeclampsia definition: 130/80 mmHg. Eur. J. Obstet. Gynecol. Reprod. Biol. 240, 322–324 (2019).

40. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in Pregnancy: Executive Summary. Obstet. Gynecol. 2013, 122, 1122–1131, doi:10.1097/01.AOG.0000437382.03963.88.

41. Matthews, D. R. et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419 (1985).

42. Phipps, E. A., Thadhani, R., Benzing, T. & Karumanchi, S. A. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat. Rev. Nephrol. 15, 275–289 (2019).

43. Mayrink, J., Costa, M. L. & Cecatti, J. G. Preeclampsia in 2018: Revisiting Concepts, Physiopathology, and Prediction. Sci. World J. 2018, 1–9 (2018).

44. Rana, S., Lemoine, E., Granger, J. P. & Karumanchi, S. A. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ. Res. 124, 1094–1112 (2019).

45. Simonds, S. E. et al. Leptin Mediates the Increase in Blood Pressure Associated with Obesity. Cell 159, 1404–1416 (2014).

46. Hall, J. E. et al. Obesity-induced Hypertension: Role of Sympathetic Nervous System, Leptin, and Melanocortins. J. Biol. Chem. 285, 17271–17276 (2010).

47. Bell, B. B. & Rahmouni, K. Leptin as a Mediator of Obesity-Induced Hypertension. Curr. Obes. Rep. 5, 397–404 (2016).

48. Dunbar, J. C., Hu, Y. & Lu, H. Intracerebroventricular Leptin Increases Lumbar and Renal Sympathetic Nerve Activity and Blood Pressure in Normal Rats. Diabetes 46, 2040–2043 (1997).

49. Shek, E. W., Brands, M. W. & Hall, J. E. Chronic Leptin Infusion Increases Arterial Pressure. Hypertension 31, 409–414 (1998).

50. Papadopoulos, D. P. et al. Human Soluble Leptin Receptor Concentration in Healthy Offspring of Hypertensive Parents. J. Clin. Hypertens. 8, 797–802 (2006).