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Original Article

Physiology Section

Time Course of Changes in Leptin Levels and their Relationships with Oxidant Status Biomarkers in Pregnant Women with Obesity

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ABSTRACT

Introduction: Changes in leptin signaling during obesity are emerging as a potential risk factor leading to pregnancy complications. Increased oxidative stress may also contribute to the adverse outcomes associated with pregnancy in obese women. Although hyperleptinemia and oxidative stress are risk factors of pregnancy complications during obesity, their relationships have not yet been clarified.

Aim: To explore the association between leptin and oxidative stress status in maternal obesity and to evaluate the prediction of oxidant biomarkers by maternal leptin in first trimester.

Materials and Methods: This longitudinal study included 60 obese pregnant women and 80 controls who were followed during the three trimesters of pregnancy. Oxidative stress parameters such as nitric oxide (NO), superoxide anion (O_2), peroxynitrite, Malondialdehyde (MDA), hydroperoxides, and Protein Carbonyl (PC) contents were measured spectrophotometrically. Serum leptin was measured by ELISA assay. Association between leptin and oxidative stress parameters was performed by multiple regression analysis.

Results: Plasma leptin (more than 40%) and oxidant marker concentrations (more than 25%) were significantly higher in obese pregnant women compared to control subjects from the first to the last trimester. Two-way repeated measures ANOVA revealed a significant effect for obesity and time on leptin and oxidant levels (F-values 10.42 to 185.25 for obesity effect and 3.01 to 49.35 for time effect; $p<0.01$). Leptin concentration was correlated to all oxidant markers ($p<0.0008$) and explained in general 22 to 52% of their variation. Leptin level measured in the 1st trimester permitted estimation of 3rd trimester hydroperoxide, O_2, NO, MDA, PC and peroxinitrite concentrations (p-value<0.0001) and accounted for 77.30 to 94.70% of their variation.

Conclusion: Obesity during pregnancy is characterised by high concentrations of leptin and oxidant markers. The leptin levels could be used to predict oxidative stress in late gestation. An early identification of women with increased risk of oxidative stress may provide a window of opportunity to improve redox status by antioxidant supplementation.

Keywords: First trimester leptin, Hydroperoxides, Malondialdehyde, Maternal obesity, Nitric oxide, Peroxynitrite, Pregnancy trimester, Protein carbonyl, Superoxide anion

INTRODUCTION

Now-a-days, several strategies are used to prevent obesity and in improving metabolic and mental status [1]. In addition, prevention of obesity during pregnancy is important to reduce mortality and morbidity in both mother and offspring [2]. Obesity has an adverse impact on maternal and neonatal outcome as it is associated with several conditions such as gestational diabetes, hypertension, pre-eclampsia, congenital anomalies, foetal macrosomia, caesarean delivery and neonatal death [2,3]. Maternal obesity was found to be associated with dyslipidemia, hyperinsulinemia, vascular dysfunction, and low-grade chronic inflammation [2-4].

Adipokines have been implicated in the development of many disorders associated to obesity such as diabetes and cardiovascular disease [5]. Leptin plays an important role in the regulation of maternal metabolic homeostasis, in placentation and maternal-foetal exchange processes [6]. At the end of pregnancy, leptin resistance appears to facilitate nutrient transfer to the foetus. Changes in leptin signaling during obesity are thus emerging as a potential risk factor leading to pregnancy complications [1,7]. Leptin acts as a pro-inflammatory cytokine that might have a role in the development of obesity related complications [7,8]. High leptin levels were noted in obese women compared to controls, with a slight increase during pregnancy [9,10]. Maternal obesity during pregnancy increases leptin resistance since elevated leptin levels are not sufficient to prevent disturbances in energy balance [6,7]. In fact, leptin resistance is apparent in both obesity [11,12] and pregnancy [6,13] and it can be accentuated by the combination of these two factors. In addition, high leptin levels are observed with several risk factors related to obesity including hyperglycaemia, hyperlipidemia and inflammation [14]. Moreover, low antioxidant combined with increased oxidative stress and inflammation may also contribute to the adverse outcomes associated with pregnancy in obese women [15,16].

Oxidative stress is considered as a risk factor during pregnancy [15,17,18]. In addition, the presence of oxidative stress biomarkers has been widely demonstrated in maternal obesity [16,19]. During pregnancy, high placental metabolic activity and high rate of oxygen consumption generate oxidative stress. Both obesity and gestation are thus characterised by oxidative stress. Leptin is a factor increasing free radical production and oxidative stress in obesity [20]. Some previous studies have established relationships between hyperleptinemia and oxidative stress biomarkers in obese subjects [19-21]. Although the relationship between metabolic risk factors and leptin have been widely documented, evidence of whether there is any advantage in assessing leptin in term of the prediction of maternal obesity-related oxidative stress is less understood. In addition, since maternal obesity is associated with oxidative stress in the first trimester [22], the relationship between maternal leptin concentrations in the first trimester and gestational evolution of oxidative stress is unclear. In fact, the information regarding the influence of first trimester-leptin levels on oxidant markers during maternal obesity is scarce. Previous results supported altered oxidant/antioxidant status in obese women during the end of pregnancy [23,24]. However, time course changes of oxidant/

antioxidant balance and leptin concentrations and their relationships during pregnancy were not investigated in these obese women.

To extend these previous findings, the present study was conducted to analyse the association between hyperleptinemia and oxidative stress in maternal obesity. Firstly, the possible relationships of leptin with various oxidative stress parameters were investigated in obese and healthy mothers at three trimesters of pregnancy, and secondly the prediction of oxidant biomarkers by maternal leptin in the first trimester was evaluated. This approach may encourage clinicians to introduce first trimester leptin levels as a predictive biomarker of oxidative stress in obese pregnancy. Recommendations are to develop therapeutic strategies including antioxidant supplementation in early pregnancy aimed at reducing excessive oxidative stress and its adverse effects.

MATERIALS AND METHODS

This longitudinal study was conducted from July 2017 to March 2019, and included 140 pregnant women at 1st trimester of pregnancy followed until delivery at the Obstetrics and Gynaecology department of Tlemcen Hospital, Tlemcen city (West Algeria). The protocol was approved by the Tlemcen Hospital Committee for Research on Human Subjects (CNEPRU I02020110069). To obtain a power of 85% sample size calculator (Statistical solutions, Sigma) was used and a total of 80 normal weight and 60 obsese women were selected.

The inclusion criteria for control group were a pre-pregnancy BMI between 19 and 25 kg/m^2, age between 25 and 35 years, full-term pregnancies (>37 weeks), uncomplicated singleton pregnancies and normal delivery. The exclusion criteria included a history of chronic diseases, gestational diabetes, hypertension, eclampsia, infections or foetal anomalies. For obese group, the inclusion criteria were a pre-pregnancy BMI ≥ 30 kg/m^2, age and gestational age matched with control group, uncomplicated singleton pregnancies and normal delivery. Exclusion criteria also included chronic diseases and pregnancy complications. The women were examined for gestational diabetes as per WHO criteria [25] and glucose tolerance test levels were normal during third trimester and within 48 hour of delivery. The participants were informed and written consent was obtained.

Blood Samples

Fasting maternal blood samples were obtained from the arm veins of the mothers during routine exam (1st and 2nd trimester), and at the time of delivery (3rd trimester). In EDTA tubes blood samples were collected and after centrifugation plasma was seperated and biochemical parameters, hormones and plasma oxidative stress markers were analysed. The left out erythrocytes were washed and haemolysed by the addition of cold water.

Biochemical Parameters

Enzymatic methods (Kits Sigma Chemical Company, St Louis, Mo, USA) were used to determine Plasma glucose, total cholesterol, HDL- cholesterol and triglycerides.

Determination of Leptin and Insulin

Leptin and insulin were determined by ELISA sandwich assays for human leptin and insulin respectively (Crystal Chem kits, USA). These assays utilise a specific antibody immobilised onto the microplate wells and an antibody labeled with HRP enzyme to achieve a specific assay. The absorbance at 450 nm is proportional to the concentrations of leptin or of insulin. A standard curve was constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

Determination of the Oxidant Status Markers

The Nitric Oxide (NO) concentrations were determined by the nitrite/nitrate assay kit (Sigma Aldrich kit, St. Louis, MO), using Griess reaction. As described by Auclair C and Voisin E [26], erythrocyte superoxide anion levels were determined using the superoxide anion mediated Nitro Blue Tetrazolium (NBT) reduction to monofarmazan, a chromophore that absorbs at 550 nm. Erythrocyte peroxynitrite levels were determined according to the procedure described by Beckman JS et al., cited by Van Uffelen BE et al., [27,28]. The method consists of a spectrophotometric measurement of peroxynitrite mediated nitration of phenol.

Hydroperoxides, a widely used marker for lipid peroxidation, were measured spectrophotometrically using ferrous oxidation in xylenol orange assay (PeroxiDetect kit; Sigma, St. Louis, MO). Erythrocyte MDA, one of the end products of lipid peroxidation, was determined by the well known TBARS assay (Lipid Peroxidation (MDA) Assay Kit; Sigma Aldrich kit, St. Louis, MO). Erythrocyte carbonyl proteins as biomarker of protein oxidation were evaluated by 2,4-dinitrophenylhydrazine reaction (Sigma Aldrich kit, St. Louis, MO).

STATISTICAL ANALYSIS

The results were presented as means and standard deviations. The results were tested for normal distribution using the Shapiro-Wilk test. Differences between the groups in baseline characteristics were evaluated with unpaired t-tests. Two-way ANOVA with repeated measurements was used to evaluate differences in leptin and oxidative biomarkers between normal weight and obese groups during the 1st, 2nd and 3rd trimesters. When ANOVA indicated a significant difference for a particular factor, Bonferroni post-hoc analysis was performed to identify the source of the difference. Multiple regression analysis was employed to determine if leptin was a predictor variable of oxidant biomarkers through pregnancy. A second multiple regression analysis was performed with dependent variables (markers of oxidant status in the 3rd trimester) and independent variable (leptin concentration in the first trimester). The values were considered as statistically significant if $p<0.05$. All tests were performed using STATISTICA 10 software (StatSoft, Tulsa, OK, USA).

RESULTS

A significant increase in plasma glucose, insulin, total cholesterol, and triglyceride levels was clearly evidenced in the obese mothers compared to control ones [Table/Fig-1].

Characteristics	Control women	Obese women	p-values
Number	80	60	
Age (years)	28±3	31±3	0.145
Pre-pregnancy BMI (kg/m^2)	22±2.50	34.50±2.25	0.006*
Systolic blood pressure (mmHg)	11.75±1.22	12±0.80	0.117
Diastolic blood pressure (mmHg)	7.54±1.11	7.75±1.25	0.156
Gestational age (weeks)	39±1	40±2	0.271
Number of gestation	4±1	4±2	0.208
Parity	3±1	3±1	0.224
Glucose (mg/dL)	112±7	140±10	0.007*
Insulin (ng/mL)	1.15±0.10	2.68±0.15	0.006*
Total Cholesterol (mg/dL)	218±10	280±11	0.008*
HDL-Cholesterol (mg/dL)	62±5	60±4	0.144
Triglycerides (mg/dL)	210±15.35	295±16.57	0.007*

[Table/Fig-1]: Maternal characteristics.
Values are means±SD. BMI: Body mass index (weight/height^2). Significant differences between obese and control groups are indicated as: *p<0.01

Leptin concentrations of the two populations are shown in [Table/Fig-2]. Plasma leptin concentrations were significantly higher in obese mothers compared to control subjects all along the pregnancy. Leptin concentrations increased from early to late gestation in the two groups. Two-way repeated measures ANOVA revealed a significant effect for obesity on plasma leptin levels (F-values 108; p<0.0001).

There were also significant effects of time (along the pregnancy) on these leptin concentrations (F-values 24.09; p<0.001). However, leptin was more affected by obesity than time.

				Two-way repeated ANOVA (F-values)	
Parameters	**1st trimester**	**2nd trimester**	**3rd trimester**	**Ob**	**Ti**
Leptin (ng/mL)					
Control	11.54±1.15	18.56±2.25†	28.78±1.54†$		
Obese	30.26±2.22*	45.80±3.00*†	56±3.26*†$	108c	24.09b

[Table/Fig-2]: Leptin concentrations at different trimesters of pregnancy in control and obese women.
Values are means±SD; Ob: effect of obesity; Ti: effect of time. Statistical analysis: Two-way ANOVA followed by Bonferroni post-hoc analysis. b, c Represent the significant effect in two-way repeated ANOVA: p<0.001, and p<0.0001 respectively. *Significant differences between obese and control women; p<0.05; †Significant differences between 2nd or 3rd trimester and 1st trimester; p<0.05; $Significant differences between 3rd trimester and 2nd trimester; p<0.05

Oxidant status alterations in obese mothers is shown in [Table/Fig-3] and were marked by a significant increase in erythrocyte concentrations of nitric oxide, anion superoxide and peroxynitrite concentrations compared to control values in the three trimesters. Furthermore, MDA and HP as lipid peroxidation biomarkers and CP as protein oxidation markers were higher in obese mothers than in controls, whatever the trimester of the pregnancy. All oxidant parameters increased from the first to the last trimester of pregnancy in both mothers groups. Indeed, there were significant effects (two-way repeated measures ANOVA) of obesity and time on HP, MDA, CP and peroxynitrite levels. However, NO and anion superoxide concentrations were only affected by obesity.

				Two-way repeated ANOVA (F-values)	
Parameters	**1st trimester**	**2nd trimester**	**3rd trimester**	**Ob**	**Ti**
NO (µmol/L)	-	-	-		
Control	1.56±0.42	2.31±0.65	3.24±0.41†$		
Obese	3.26±0.57*	4.63±0.62*†	5.55±0.72*†	36.45b	4.26
$O_2^{\cdot-}$ (µmol/L)	-	-	-		
Control	9.44±1.29	11.56±1.55	12.62±1.43†		
Obese	13.00±2.03*	18.79±1.93*†	19.84±1.62*†	11.95a	3.01
HP (µmol/L)	-	-	-		
Control	1.88±0.12	2.04±0.15	2.46±0.18†$		
Obese	2.66±0.21*	3.75±0.24*†	5.05±0.21*†$	185.25c	31.62b
MDA (µmol/L)	-	-	-		
Control	1.56±0.10	1.88±0.22	2.76±0.23†$		
Obese	2.30±0.23*	2.89±0.14*†	4.55±0.25*†$	82.55c	49.35b
CP (µmol/L)	-	-	-		
Control	1.76±0.34	1.80±0.22	2.68±0.31†$		
Obese	3.06±0.23*	2.84±0.11*	3.88±0.20*†$	10.42a	6.94a
$ONOO^-$ (µmol/L)	-	-	-		
Control	527±33	584±54	658±67†$		
Obese	612±25*	762±43*†	890±41*†$	83.24c	13.33a

[Table/Fig-3]: Oxidant markers in control and obese women during pregnancy.
Values are means±SD; CP: Carbonyl proteins; HP: Hydroperoxides; MDA: Malondialdehyde; $O_2^{\cdot-}$: Superoxide anion; ONOO-: Peroxynitrite; NO: Nitric oxide; Ob: Effect of obesity; Ti: Effect of time. Statistical analysis: Two-way ANOVA followed by Bonferroni post-hoc analysis. a, b, c Represent the significant effect in two-way repeated ANOVA: p<0.01, p<0.001, and p<0.0001 respectively. *Significant differences between obese and control women; p<0.05. †Significant differences between 2nd or 3rd trimester and 1st trimester; p<0.05. $Significant differences between 3rd trimester and 2nd trimester; p<0.05

To clarify the influence of leptin on oxidant markers through the pregnancy, multiple regression analysis [Table/Fig-4] was performed with independent variable (leptin) and dependent variables (oxidant parameters). Leptin concentration was a predictor of all oxidant markers (NO, $O_2^{\cdot-}$, HP, MDA, CP, peroxynitrite) and explained 22 to 52% of their variation for the first trimester, 22.70 to 46% for the second trimester and 28.80 to 35.40% for the last trimester.

The second model of multiple regression analysis [Table/Fig-5] was performed with only first trimester leptin concentration as independent variable and third trimester oxidant parameters as dependent variables. The results showed that leptin concentrations measured in the 1st trimester permitted estimation of 3rd trimester oxidant parameters (hydroperoxide, $O_2^{\cdot-}$, NO, MDA, PC and peroxinitrite) and accounted for 77.30 to 94.70% of their variation.

Dependent variables	Leptin (independent variable)		
1st trimester	**β (SE)**	**p-values**	**R^2**
NO (µmol/L)	0.675 (0.141)	0.0004	0.455
$O_2^{\cdot-}$ (µmol/L)	0.654 (0.082)	0.0004	0.427
HP (µmol/L)	0.687 (0.111)	0.0004	0.472
MDA (µmol/L)	0.721 (0.075)	0.0003	0.519
CP (µmol/L)	0.643 (0.108)	0.0004	0.413
$ONOO^-$ (µmol/L)	0.467 (0.067)	0.0008	0.221
2nd trimester			
NO (µmol/L)	0.581 (0.073)	0.0006	0.337
$O_2^{\cdot-}$ (µmol/L)	0.643 (0.077)	0.0004	0.413
HP (µmol/L)	0.679 (0.052)	0.0004	0.461
MDA (µmol/L)	0.574 (0.034)	0.0006	0.329
CP (µmol/L)	0.567 (0.054)	0.0006	0.321
ONOO- (µmol/L)	0.477 (0.037)	0.0008	0.227
3rd trimester			
NO (µmol/L)	0.588 (0.072)	0.0006	0.346
$O_2^{\cdot-}$ (µmol/L)	0.575 (0.108)	0.0006	0.331
HP (µmol/L)	0.595 (0.089)	0.0006	0.354
MDA (µmol/L)	0.592 (0.044)	0.0006	0.350
CP (µmol/L)	0.538 (0.064)	0.0007	0.289
$ONOO^-$ (µmol/L)	0.537 (0.105)	0.0007	0.288

[Table/Fig-4]: Multiple regression analysis with leptin as independent variable and with oxidant markers as dependent variables.
β (SE) indicates regression coefficient (standard error); R^2: the coefficient of determination, represents the percentage of the variation in the dependent variable explained by the independent variable in the model

Dependent variables	Leptin- 1st trimester (independent variable)		
3rd trimester	**β (SE)**	**p-values**	**R2**
NO (µmol/L)	0.967 (0.112)	0.0001	0.935
$O_2^{\cdot-}$ (µmol/L)	0.954 (0.087)	0.0001	0.911
HP (µmol/L)	0.973 (0.079)	0.0001	0.947
MDA (µmol/L)	0.943 (0.069)	0.0001	0.889
CP (µmol/L)	0.879 (0.077)	0.0001	0.773
ONOO- (µmol/L)	0.923 (0.061)	0.0001	0.852

[Table/Fig-5]: Multiple regression analysis with leptin-1st trimester as independent variable and with oxidant markers-3rd trimesters as dependent variables.
β (SE) indicates regression coefficient (standard error); R2: the coefficient of determination; represents the percentage of the variation in the dependent variable explained by the independent variable in the model

DISCUSSION

This study provides evidence that obesity alters the oxidant status in obese mothers throughout pregnancy. High leptin levels were observed in these obese mothers and were correlated with all oxidant markers in the three trimesters of pregnancy. Indeed, leptin concentrations in the first trimester were a strong predictor of oxidative stress in late gestation. During pregnancy, hormonal and metabolic changes in the first trimester directly affect gestation progression [29]. Proper timing of endocrine events has been previously mentioned during different situations, including even the effects of timing exercise on hormonal secretion [30].

Authors found higher leptin levels in obese mothers in the three trimesters of pregnancy [31,32] but increased at a significantly lower rate across gestation, compared to normal weight [33]. This is in

accordance with findings of the present study. In fact, Hendler I et al., found that leptin levels increased with maternal BMI. Patients with overweight and obese women had increased leptin levels (+50%) compared with normal weight women [31]. Yang MJ noted a good correlation between maternal leptin and BMI in the three trimesters ($p < 0.007$) of pregnancy [32]. Castellano Filho DS et al., found a progressive increase in maternal weight gain and in leptin levels during pregnancy in both control and obese groups [33]. However, the increase was significantly higher in the non-overweight patient group. The authors explained that the greater increase in leptin levels in non-overweight pregnant women is associated to the higher weight gain in this group compared to overweight/obese women [33].

Previous studies have shown that high leptin levels in obese mothers were associated to the developmental programming of cardiometabolic risk in the offspring [34,35]. Hence, several previous studies proposed that leptin concentration may represent a useful tool for evaluating pregnancy risks [6,36].

Obese pregnant women have increased oxidative stress, compared with healthy pregnant women. It was reported in various studies that obesity is linked with oxidative stress due to rate of free radical formation is increased and there is scarcity of antioxidant defenses. [15,16,19,23]. In the present study, obese mothers showed high levels of oxidant markers (nitric oxide, superoxide anion, peroxynitrite, hydroperoxide, malondialdehyde and carbonyl proteins) compared to controls in the three trimesters of pregnancy. The authors have previously reported high oxidative stress in obese pregnancy at delivery [23,24]. The present findings give an additional information on the presence of oxidative stress from the first to the end of pregnancy in obese mothers. The results of this study suggested that obese women had a pre-existing oxidative stress which is enhanced during pregnancy.

Furthermore, the present results showed that leptin was positively correlated with oxidant markers in pregnant women during the three trimesters. Obese mothers with hyperleptinemia were also subjected to an enhanced oxidant production. Several previous studies suggested an association between leptin and oxidative stress markers [11,19,20,36,37]. A high correlation of leptin level with lipid profile and oxidative stress levels was reported during obesity [37]. Leptin induced oxidative stress via increased fatty acid oxidation [38]. Leptin promotes oxidative stress by increasing phagocytic activity of macrophages, inducing pro-inflammatory cytokine synthesis, and activating several cells (T-cells, monocytes, neutrophils, and endothelial cells) [39]. It has been shown that hyperleptinemia plays a key role in the formation of lipid peroxides thus mediating oxidative stress [40].

In the present study, leptin level could be used as a predictor for oxidative stress in late gestation. To our knowledge, this relationship has not been presented in the literature yet, pointing the need of specific interventions to obese mothers with hyperleptinemia. An early identification of women with increased risk of oxidative stress may provide a window of opportunity to improve redox status by antioxidant supplementation.

Limitation(s)

One limitation was the small sample size. Second, BMI was recorded at a single time point and gestational weight gain was not considered. In addition, energy intake and lifestyle factors were not reported. It remains possible that confounder variables are mediating the relationship between leptin and oxidative stress during obese pregnancy. Further studies are needed with large number of sample size, considering potential confounders such as gestational weight gain, energy intake and lifestyle factors.

CONCLUSION(S)

Obesity during pregnancy is characterised by high leptin concentrations and oxidant markers from the first to the last trimester. Leptin concentrations could be used as a biomarker of oxidative stress progression and severity during pregnancy. As oxidative stress is closely related to pregnancy complications, the results indirectly reinforce the importance of maternal redox control during pregnancy to avoid adverse outcomes to mother and their newborns. Therefore, determination of leptin levels in obese pregnant women in early gestation may guide to the risk of developing excessive oxidative stress, and may reinforce an antioxidant intervention from the beginning of pregnancy.

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