http://rjdnmd.org
Rom J Diabetes Nutr Metab Dis. **26**(2):129-143
doi: 10.2478/rjdnmd-2019-0014

INTERRELATIONS BETWEEN INFLAMMATORY AND OXIDATIVE STRESS BIOMARKERS IN OBESE WOMEN WITH TWO COMPLICATIONS (HYPERTENSION, DIABETES)

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received: February 01, 2019 *accepted*: June 10, 2019
available online: June 30, 2019

Abstract

Background and aims: *Interaction between oxidative stress and inflammation has not been comprehensively investigated in the association obesity – diabetes - hypertension. Our aim was to investigate interrelations between inflammatory and oxidative stress biomarkers in obese women with two complications (hypertension, type 2 diabetes).* ***Material and Methods:*** *54 obese patients without complications, 46 diabetic patients with obesity, 48 hypertensive diabetic obese women, and 120 healthy controls were recruited from the department of nuclear medicine (Algeria). Inflammatory and oxidative stress biomarkers were assayed by appropriate methods.* ***Results:*** *Inflammatory markers were significantly higher in all obese groups compared to controls. Elevated pro-oxidants and decreased antioxidant markers were noted in obese women. These alterations were accentuated when obesity was associated with hypertension and diabetes. A positive interrelationship between inflammatory mediators and oxidative status, and a negative one with antioxidants were noted during obesity. Hypertension and diabetes enhanced these correlations. Leptin, C-reactive protein, catalase, superoxide dismutase, ion superoxide, peroxynitrite were found to be the best inflammatory and oxidative stress biomarkers that can predict diabetes and hypertension in obese women.* ***Conclusions:*** *oxidative stress and inflammation were intimately interconnected in women obesity associated with diabetes and hypertension.*

key words: *Hypertension, inflammation, obesity, oxidative stress, type 2 diabetes mellitus.*

Background and aims

Obesity is characterized by a chronic low-grade inflammation due to the expansion of adipocytes (hypertrophy), and by adipogenesis (hyperplasia) of adipose tissue with high pro-inflammatory and low anti-inflammatory

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adipokine expression [1]. It has been shown that obese individuals with high levels of pro-inflammatory cytokines, including interleukins (IL-6), leptin, TNF-α and CRP, are *most likely to develop* metabolic syndrome [2,3]. Currently, it is widely admitted that white adipose tissue (WAT) is, at the same time, a metabolically dynamic *organ* that *stores* excess *energy* and an endocrine organ that releases different adipokines that are involved in the regulation of *energy metabolism* [4]. The pathogenesis of obesity and insulin resistance can certainly be engendered by altered production of these adipokines as a result of WAT dysfunction and high adiposity [5].

In recent years, several epidemiological investigations showed a strong relationship between systemic vascular inflammation and hypertension, particularly in the presence of metabolic risk factors, such as obesity, dyslipidemia, and diabetes mellitus [3,6].

It has been established that *inflammation* occurs as a consequence of *oxidative stress which* increases in obese individuals. Today, it is extensively accepted that oxidative stress is one of the primary factors that triggers and maintains the inflammatory responses that are encountered within obesity and associated co-morbidities, such as diabetes, hypertension and cardiovascular disease [6,7]. In addition, oxidative stress has been suggested to be a common pathway for the pathogenesis of complications in obesity [8]. Several mechanisms can contribute to oxidative stress in obesity, including hyperglycemia, hyperlipidemia, hyperleptinemia, mitochondrial and peroxisomal oxidation of fatty acids with reactive ROS generation and low antioxidant defenses [9].

Several previous studies have reported that oxidative stress and inflammation are common pathways for the pathogenesis of complications in obesity [8,10,11]. However, to date, it is not known with certainty what enhances the oxidative stress and the inflammatory process in obesity related complications. The combination of several complications might culminate metabolic alterations. Nevertheless, recent studies indicated that being overweight or obese is associated with better outcomes in patients with established chronic diseases, supporting the presence of obesity paradox [12]. Even so another study revealed that the metabolically healthy obesity phenotype presents a risk of type 2 diabetes [13].

Indeed, the interaction between oxidative stress and inflammation has not been comprehensively investigated, especially when hypertension is found in the presence of obesity and diabetes. Furthermore, a wide number of biomarkers are used in previous studies and many of these biomarkers are interrelated in how they play a role in the development of obesity related complications. Correlations between biomarkers would be then helpful to assess at risk patients. With early detection, early intervention is possible and could be effective in reducing harmful effects.

Women are at greater risk for obesity than men, and they also appear to be more exposed to the risk for obesity related complications. Indeed, focus on obesity and its complications in women is an important consideration because they can have an adverse impact on health at each stage of a woman's life, affecting their reproductive health, or their pregnancies and their offspring [14,15]. To our knowledge, no studies have examined the potential relationship between inflammatory and oxidative stress biomarkers in obesity associated to two complications, diabetes and hypertension in women. We hypothesized that obesity would be independently associated with levels of oxidative stress and inflammation, and that diabetes and

hypertension would be significant burden. The present work aims at investigating the interrelations between inflammatory markers and oxidative stress in obese women with two complications, hypertension and type 2 diabetes.

Material and Methods

Subjects

268 premenopausal women were recruited from the Department of Nuclear Medicine, at the University Hospital of Tlemcen (Algeria), with main criteria including the *body mass index* (BMI) 18.5-24.9 Kg/m^2 or ≥ 30 Kg/m^2, and age ranging between 40 and 50 years. Different groups were selected by physicians, namely obese women with previously diagnosed high blood pressure and diabetes, obese women with antecedently diagnosed type 2 diabetes and receiving appropriate treatment, obese women without complications, and non-obese women (control group). The women were divided into four groups, depending on their associated health problems and their body mass index (BMI). The first group included 120 control women, not having any disease and not taking any medication that is likely to influence the lipid metabolism or vitamin supplements and having a normal weight (BMI between 18.5 and 24.9 Kg/m^2). The second group comprised 54 obese women without any other *pathology* (BMI ≥ 30 Kg/m^2). The third group consisted of 46 obese women with type 2 diabetes (diagnosed by means of the fasting blood glucose test with a level of 7 mmol/L or more, glycated hemoglobin test and oral glucose tolerance test). The fourth group included 48 obese women with hypertension and diabetes (with systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure (DBP) > 90 mmHg). Physicians examined all participants; their medical history was taken to collect information about general condition and current medications if any. *All participants gave their* written permission to be part of the *study*; they were given pertinent information to make an *informed consent* to *participate* to this study. The study was approved by the Ethics Committee of the University Hospital of Tlemcen (Western Algeria). The features of the patients are summarized in Table 1.

Blood samples

Blood samples were taken in the morning from the arm veins under fasting conditions and placed in heparinized tubes. After centrifugation, plasma was used for the assays of glucose, lipids (total cholesterol and triglycerides), vitamins (A, E and C), conjugated dienes, C-reactive protein, interleukins (2 and 6), leptin, and tumor necrosis factor α (TNF-α). An isotonic saline solution was used to wash the remaining erythrocytes three times; these were then hemolyzed by adding cold distilled water (1/3). The cell detritus were eliminated by centrifugation (2000g x15 min). The hemolysates were assayed for antioxidant enzyme activities (catalase and SOD), reduced glutathione (GSH), peroxynitrite, nitric oxide (°NO), ion superoxide ($O2^{\circ-}$), hydroperoxides and carbonyl proteins determinations.

Identification of biochemical and inflammatory markers

Enzymatic colorimetric methods were employed to determine the concentrations of plasma glucose, triglycerides and total cholesterol, using the Sigma- Aldrich Kits. Leptin (Human Leptin, R&D Systems, Minneapolis, MN, USA), interleukin-6 (Human IL-6, R&D Systems, Minneapolis, MN, USA), interleukin-2 (Human IL-2, R&D Systems, Minneapolis, MN, USA), high sensitive transforming growth factor-α (Human TNF-α/TNFSF1A, R&D Systems, Minneapolis, MN, USA) and high sensitive C-reactive protein

(hsCRP, Immundiagnostik AG, Bensheim, Germany) were identified by means of the commercial ELISA sets, in accordance with manufacturers' instructions.

Identification of biomarkers of oxidative stress

Spectrophotometric analysis of hydrogen peroxide decomposition rate, at 240 nm, was conducted to evaluate erythrocyte catalase (CAT EC 1.11.1.6) activity according to Sigma Aldrich kit (St. Louis, MO). The Dojindo's highly water-soluble tetrazolium salt was also employed to assess erythrocyte superoxide dismutase (SOD EC 1.15.1.1) activity (Sigma Aldrich kit, St. Louis, MO). A colorimetric method, using Ellman's reagent, and based on the reduction of 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) by GSH to generate colored 2-nitro-5-thiobenzoic acid (TNB) was employed to determine erythrocyte reduced glutathione (GSH) levels according a Sigma Aldrich kit (St. Louis, MO). Plasma vitamin C contents were determined by a coupled enzyme reaction, which results in a colorimetric product according a Sigma Aldrich kit (St. Louis, MO). The reverse phase HPLC was implemented to determine plasma α-tocopherol (vitamin E) and retinol (vitamin A). Vitamin E was detected by means of a UV detector at 292 nm, and vitamin A at 325 nm. The nitric oxide (°NO) concentration was measured by the nitrite/nitrate assay kit (Sigma Aldrich kit, St. Louis, MO), using Griess colorimetric method. A method, based on the reduction of nitroblue tetrazolium (NBT) to monoformazan by $O2^{\circ-}$ [16], was applied to quantify the concentration of erythrocyte superoxide ion. The procedure based on the peroxynitrite-mediated nitration of phenol, described by Beckman et al. [17] and reported by Van Uffelen et al. [18], was employed to determine erythrocyte peroxynitrite contents. Erythrocyte carbonyl proteins, which are protein oxidation markers, were investigated by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine resulting in the formation of stable dinitrophenyl hydrazine adducts (Sigma Aldrich kit, St. Louis, MO). Erythrocyte hydroperoxydes, which are lipid peroxidation markers, were measured by the ferrous ion oxidation-xylenol orange assay (Fox2) in combination with a specific ROOH reductant, triphenylphosphine (TPP) using a PeroxiDetect kit (Sigma, St. Louis, MO) according to manufacturer's instructions. The procedure suggested by Esterbauer et al. [19] was employed to check the susceptibility of LDL to in vitro oxidative stress, which was initiated by adding 10 μM of $CuSO_4$ at 37°C. The kinetics of LDL oxidation was progressively monitored through the measurement of conjugated diene formation, at 234 nm. Based on the experimental graphs of oxidation kinetics, the following parameters were evaluated, namely the lag time (Tlag, min), which represents the resistance to oxidation and is defined as the intercept of the straight lines derived from the lag phase and the propagation phase; the Tmax (min), which is the time needed to reach maximal amounts of conjugated dienes formed; the maximal rate of conjugated diene production (DICm, μmol/L), which is determined from the slope of the maximum absorbance curve; the initial rate of conjugated dienes (DICi,μmol/L), which may be determined from the absorbance, at time zero. The concentrations of initial and maximal conjugated dienes were estimated using the molar extinction coefficient, which is equal to 2.95×10^4 M^{-1} cm^{-1}. The oxidation rate (μmol/L/min) is expressed as (DICm - DICi) / (Tmax - Tlag).

Statistical analysis

The data obtained were written as mean ± standard deviation (SD). Statistica (version 4.1,

Statsoft, Paris, France) was employed to perform the statistical analysis, and all variables were checked for normal distribution by means of the Shapiro-Wilk's test. Comparison of means between obese women and control group women, for clinical and biochemical characteristics, was achieved by the Student's t-test. Moreover, the one-way analysis of variance (ANOVA) was used to find the differences within all groups, namely control, obese, obese + type 2 diabetes, obese + HTA + type 2 diabetes groups; this was then followed by the post-hoc Tukey test to classify the means, two by two, and compare them. Statistical differences between groups are represented by various letters (a, b, c ...). Furthermore, the Pearson correlation coefficients were calculated to estimate the strength and direction of any relationships between the inflammatory markers and biomarkers of oxidative stress.

The multiple regression analysis was carried out using independent predictor variables (BMI, complications, interaction between these variables) and dependent variables (biomarkers of inflammation and of oxidant/antioxidant status).

Results

Clinical and biochemical features

The Body Mass Index (BMI) was found to be remarkably greater in all obese women as compared to that of control women, as shown in Table 1. Also, the blood pressure (SBP and DBP) did not differ considerably between obese women without complications, diabetic women and those of control group. However, the SBP and DBP values were significantly elevated when obesity was associated with hypertension and diabetes.

Table 1. Clinical and biochemical characteristics of obese and control women.

Characteristics	Control Women	Obese Women	Obese Women +Type 2 diabetes	Obese Women +Type 2 diabetes +HTA
Number	120	54	46	48
Age (years)	43 ±3	45±4	44±4	46±3
BMI (Kg/m^2)	21.32±2.15	33.80±2.56**	34.27±2.62**	34.20±2.33**
SBP (mm Hg)	124.50±4.14	130.50±4.23	130.60±4.50	167.50±4.11**
DBP (mm Hg)	78.50±3.50	84.52±4.50	84.30±4.51	110.36±3.90**
Treatment	-	-	Statins or oral hypoglycemics	Telmisartan Almodipine Oral hypoglycemics
Glucose (mmol/L)	4.54±0.25	4.90±0.26	9.46±0.35**	8.87±0.47**
Cholesterol (mmol/L)	4.63±0.37	5.73±0.32*	6.66±0.54**	6.69±0.44**
Triglycerides (mmol/L)	1.28±0.30	1.91±0.22*	2.24±0.22**	2.65±0.20**

Values are means ± SD. BMI: Body mass index (weight/height^2); DBP: Diastolic blood pressure; SBP: Systolic blood pressure. Statistical comparison between obese groups (obese women without complications, obese women with type 2 diabetes, and obese women with type 2 diabetes and hypertension) and control group was performed by Student's t-test. * $P < 0.01$; ** $P < 0.001$.

Plasma glucose levels were not importantly changed in obese women without complications as compared to that of their control counterparts. However, higher plasma glucose levels were observed in obese diabetic women as well as in patients with diabetes and hypertension, as compared to other groups. Plasma total cholesterol and triglycerides levels were elevated in all obese women, regardless of the associated pathology. Moreover, the highest concentrations were detected in obese hypertensive diabetic and diabetic women.

Inflammatory and oxidative stress biomarkers

Plasma leptin and interleukin-6 levels were significantly higher in all obese women compared to controls, while interleukin-2 levels did not differ significantly between obese and control groups (Figure 1). TNF-α and CRP levels were significantly higher in obese groups compared to controls, the highest values were seen with complications.

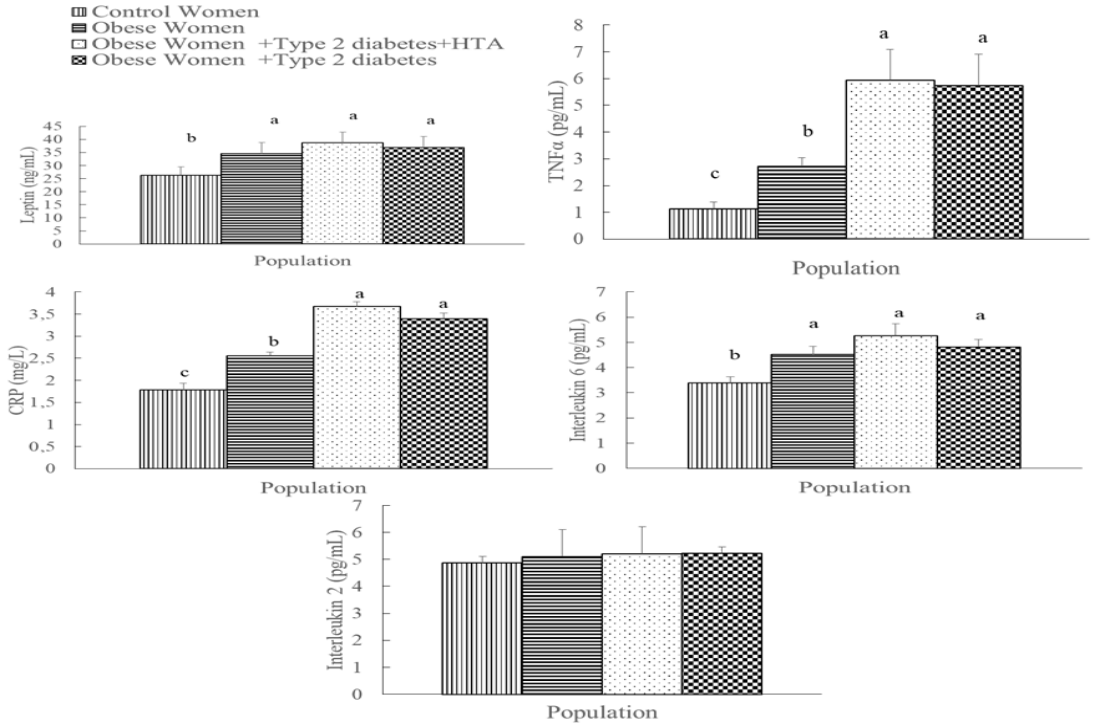

Figure 1. *Plasma inflammatory markers in obese and control women.*

Values are means ± SD. TNF-α: tumour necrosis factor alpha, CRP: C-reactive protein. Statistical comparisons between control, obese, obese with type 2 diabetes and obese with type 2 diabetes and HTA were performed by one-way ANOVA test followed by Tukey post hoc test. Values with different superscripts (a,b,c,d) are significantly different for $P < 0.05$.

Erythrocyte antioxidant enzyme activities were significantly different among the four groups under study (Table 2). The smallest values were observed when obesity/HTA/ type-2 diabetes and obesity/type-2 diabetes were combined. Reduced glutathione (GSH) was found remarkably lower in all obese patients as compared to their control counterparts. The amounts of vitamin A in obese and control groups did not differ significantly. Vitamins C and E were significantly smaller in all obese groups as compared to controls. Erythrocyte levels of nitric oxide, superoxide anion and peroxynitrite were found significantly elevated in all obese groups compared to their controls (Table 2). The highest superoxide anion and peroxynitrite values were found in obese hypertensive diabetic women. Erythrocyte protein carbonyl and hydroperoxide levels were higher in all obese women compared to controls. These levels were found to be even higher, essentially in the group of obese women with type-2 diabetes and HTA/type-2 diabetes. The initial rate of conjugated dienes and oxidation rate were considerably increased in all obese groups as compared to control group; the highest values were encountered in obese diabetic women with and without hypertension.

Table 2. Oxidative stress biomarkers in obese and control women.

	Control Women	Obese Women	Obese Women +Type 2 diabetes	Obese Women +Type 2 diabetes+HTA	*P* (ANOVA)
Catalase (U/min/mL)	89.96±3.85[a]	64.34 ±3.22[b]	52.22±3.14[c]	40.75±2.50[d]	0.001
SOD (µmol/L /min)	287±15.40[a]	245±14.60[b]	229.15±15.20[c]	200.47±11.06[d]	0.005
GSH (mmol/L)	1.85±0.05[a]	0.45±0.04[b]	0.38±0.03[b]	0.37±0.04[b]	0.006
Vitamin A (µmol/L)	13.15±2.80	12.68±2.21	11.83±2.43	12.49±1.53	0.155
Vitamin C (µmol/L)	47.35±2.30[a]	29.66±2.14[b]	27.75±2.49[b]	27.25±2.50[b]	0.008
Vitamin E (µmol/L)	23.87±2.17[a]	15.75±1.74[b]	14.20±1.17[b]	14.16±1.84[b]	0.006
°NO (µmol/L)	37.16±3.45[b]	62.14±3.25[a]	66.12±3.77[a]	67.53±3.74[a]	0.007
O2°- (µmol/L)	50.24±3.88[d]	75.37±2.64[c]	84.25±2.70[b]	95.82±3.50[a]	0.005
peroxynitrite (nmol/L)	0.66 ± 0.04[d]	1.25 ± 0.03[c]	1.85±0.03[b]	2.25 ± 0.02[a]	0.007
Carbonyl proteins (nmol/mg protein)	3.72±0.49[c]	5.96±0.27[b]	6.53±0.17[a]	7.43±0.22[a]	0.001
Hydroperoxides (µmol/L)	2.14±0.19[c]	4.38±0.20[b]	5.39±0.14[a]	6.41±0.15[a]	0.001
Conjugated dienes (µmol/L)	35.17±1.50[c]	48.65±1.27[b]	57.82±1.45[a]	58.95±1.70[a]	0.006
Oxidation rate (µmol/min/L)	26.24±1.80[c]	39.13±2.11[b]	48.68±1.50[a]	49.83±1.56[a]	0.007

Values are means ± SD. SOD: superoxide dismutase, GSH: reduced glutathione, °NO: nitric oxide, O2°$^-$: ion superoxyde. Statistical comparison between the four groups of women (control, obese, obese with type 2 diabetes, obese with type 2 diabetes and HTA) was performed by one-way ANOVA test followed by Tukey post hoc test. Values for each parameter with different superscripts (a,b,c,d) are significantly different for $P < 0.05$.

Correlations between inflammatory and oxidative stress biomarkers

Table 3 showed correlation coefficients (Pearson's) between inflammatory and oxidative stress biomarkers in obese women with different complications and in controls. In control women, leptin, interleukin-6, CRP and TNF-α concentrations were positively correlated with catalase, SOD, GSH, vitamin C, E, °NO, O2°-, hydroperoxides, carbonyl proteins, conjugated dienes, peroxynitrite and oxidation rate ($P < 0.05$). The correlations between inflammatory biomarkers and oxidant/ antioxidant status in obese women showed that leptin, interleukin-6, CRP and TNF-α were negatively correlated with antioxidant biomarkers (catalase, SOD, GSH, vitamin C and E) and positively with pro-oxidant biomarkers (°NO, O2°-, hydroperoxides, carbonyl proteins, peroxynitrite, conjugated dienes and oxidation rate) ($P < 0.01$).

The correlation between inflammatory and oxidative stress biomarkers in obese women with type 2 diabetes mellitus or with diabetes and hypertension were similar to those observed for obese women and showed that inflammatory biomarkers were correlated negatively with antioxidant biomarkers and positively with oxidant biomarkers ($P < 0.001$). We noted that in the presence of these two complications, the associations between inflammatory and oxidative stress biomarkers were more significant than those found in the presence of obesity alone.

Table 3. Correlations between inflammatory and oxidative stress biomarkers.

Parameters	Inflammatory markers	Control Women	Obese Women	Obese Women +Type 2 diabetes	Obese Women +Type 2 diabetes +HTA
Catalase	Leptin	0.25*	-0.48**	-0.49**	-0.50**
	IL-6	0.34*	-0.47**	-0.52**	-0.59**
	TNFα	0.28*	-0.50**	- 0.63**	-0.65**
	CRP	0.31*	-0.51**	-0.55**	-0.61**
SOD	Leptin	0.33*	- 0.57**	-0.52**	-0.58**
	IL-6	0.34*	-0.48**	-0.54**	-0.60**
	TNFα	0.37*	-0.55**	-0.60**	-0.61**
	CRP	0.25*	-0.52**	-0.68***	-0.73***
GSH	Leptin	0.37*	-0.53**	-0.63**	-0.60**
	IL-6	0.28*	- 0.47**	-0.58**	-0.62**
	TNFα	0.33*	-0.49**	-0.48**	-0.47**
	CRP	0.26*	-0.43**	-0.50**	-0.53**
Vitamin C	Leptin	0.27*	-0.49**	-0.53**	-0.58**
	IL-6	0.30*	-0.50**	-0.48**	-0.49**
	TNFα	0.31*	-0.47**	-0.51**	-0.55**
	CRP	0.23*	-0.55**	-0.52**	-0.61**
Vitamin E	Leptin	0.35*	-0.48**	-0.46**	-0.64***
	IL-6	0.32*	-0.50**	-0.71***	-0.73***
	TNFα	0.31*	-0.54**	-0.58**	-0.69***
	CRP	0.24*	-0.43**	-0.62**	-0.64**
°NO	Leptin	0.21*	0.48**	0.64***	0.71***
	IL-6	0.30*	0.52**	0.59**	0.65***
	TNFα	0.22*	0.50**	0.58**	0.61**
	CRP	0.25*	0.48**	0.56**	0.57**
O2°-	Leptin	0.29*	0.63**	0.56**	0.83***
	IL-6	0.28*	0.62**	0.77***	0.86***
	TNFα	0.30*	0.59**	0.69***	0.76***
	CRP	0.23*	0.54**	0.56**	0.60**
Hydroperoxides	Leptin	0.35*	0.55**	0.61**	0.65**
	IL-6	0.34*	0.51**	0.49**	0.53**
	TNFα	0.31*	0.63**	0.69***	0.72***
	CRP	0.32*	0.54**	0.55**	0.60**
Carbonyl proteins	Leptin	0.31*	0.54**	0.56**	0.58**
	IL-6	0.35*	0.51**	0.63**	0.64**
	TNFα	0.24*	0.49**	0.45**	0.46**
	CRP	0.26*	0.57**	0.61**	0.62**
Conjugated dienes	Leptin	0.41*	0.50**	0.52**	0.70***
	IL-6	0.40*	0.57**	0.60**	0.69***
	TNFα	0.21*	0.47**	0.68***	0.69***
	CRP	0.26*	0.44**	0.52**	0.57**
Oxidation rate	Leptin	0.38*	0.61**	0.71***	0.80***
	IL-6	0.31*	0.45**	0.57**	0.63**
	TNFα	0.20*	0.45**	0.68***	0.69***
	CRP	0.24*	0.42**	0.60**	0.68***
Peroxynitrite	Leptin	0.21*	0.43**	0.52**	0.71***
	IL-6	0.33*	0.54**	0.63**	0.75***
	TNFα	0.35*	0.56**	0.64**	0.73***
	CRP	0.42*	0.57**	0.90***	0.92***

Values represent correlation coefficients (R). Statistically significant: *P <0.05; **P <0.01; ***P <0.001.

Predictors of metabolic alterations

Good predictors can be determined for metabolic alterations during obesity, associated or not with complications, such as HTA and type 2 diabetes, using multiple regression analysis with independent variables or predictors (BMI, presence of complications and interactions) and dependent variables (biomarkers of inflammation and oxidant/antioxidant status) (Table 4). In this analysis, BMI and complications were significant predictors of leptin, CRP, TNF-α and IL-6 levels. The interaction of these variables increases the prediction force and explains 43 % to 78.50% of the variation of these parameters. BMI and complications were significant predictors of antioxidant biomarkers (vitamin C, E, catalase, SOD, GSH) and also of oxidant biomarkers (conjugated dienes, oxidation rate, carbonyl proteins, hydroperoxides, O2°-, °NO and peroxynitrite). The interaction of these predictors explains 27.00% to 82.60% of the variation of these parameters of the redox balance.

Table 4. Multiple regression analysis.

Dependent variables	Independent variables		
	BMI	Complications	Interaction
Leptin *B* (ES) *P* R^2	 0.412(0.067) 0.006 -	 0.427(0.101) 0.006 -	 0.001 0.780
IL-6 *B* (ES) *P* R^2	 0.353(0.064) 0.008 -	 0.427(0.107) 0.020 -	 0.006 0.430
TNF-α *B* (ES) *P* R^2	 0.250(0.082) 0.030 -	 0.419(0.071) 0.006 -	 0.005 0.520
CRP *B* (ES) *P* R^2	 0.414(0.107) 0.007 -	 0.531(0.100) 0.003 -	 0.001 0.785
Vitamine E *B*(ES) *P* R^2	 -0.690(0.104) 0.006 -	 -0.680(0.074) 0.006 -	 0.006 0.401
Catalase *B*(ES) *P* R^2	 -0.447(0.079) 0.005 -	 -0.572(0.153) 0.006 -	 0.001 0.817
SOD *B*(ES) *P* R^2	 -0.271(0.075) 0.007 -	 -0.688(0.123) 0.004 -	 0.001 0.826
Conjugated dienes *B*(ES) *P* R^2	 0.258(0.026) 0.005 -	 0.629(0.051) 0.003 -	 0.006 0.450

Table 4. *Continued*.

Dependent variables	Independent variables		
	BMI	Complications	Interaction
Oxydation rate *B*(ES) *P* R^2	 0.344(0.062) 0.003 -	 0.346(0.121) 0.005 -	 0.005 0.555
Carbonyl proteins *B*(ES) *P* R^2	 0.311(0.053) 0.006 -	 0.852(0.063) 0.003 -	 0.005 0.530
$O2^{\circ -}$ *B*(ES) *P* R^2	 0.251(0.097) 0.008 -	 0.689(0.168) 0.002 -	 0.002 0.790
Hydroperoxides *B*(ES) *P* R^2	 0.259(0.066) 0.007 -	 0.719(0.107) 0.005 -	 0.006 0.460
°NO *B*(ES) *P* R^2	 0.243(0.091) 0.009 -	 0.663(0.168) 0.004 -	 0.006 0.470
GSH *B* (ES) *P* R^2	 -0.316(0.091) 0.007 -	 -0.298(0.064) 0.011 -	 0.006 0.440
Vitamin C *B* (ES) *P* R^2	 -0.327(0.057) 0.006 -	 -0.256(0.079) 0.023 -	 0.010 0.270
Peroxynitrite *B* (ES) *P* R^2	 0.250(0.090) 0.007 -	 0.481(0.113) 0.006 -	 0.002 0.772

B (ES) are the correlation coefficients (standard error) of each independent variable with the dependent variable. R2 is the coefficient of determination; it provides the percentage of variance expressed by all variables. Relationships were significant for $p< 0.05$.

Discussion

Our present investigation added more confirmations to closely link inflammatory markers with oxidative stress in obese women with two complications, hypertension and type 2 diabetes. Firstly, obese women presented many metabolic alterations, including inflammation and oxidative stress, which were intensified and aggravated in the presence of hypertension and type 2 diabetes. Secondly, the association obesity – complication was a strong predictor of inflammatory and oxidative stress biomarkers in women. Thirdly, some biomarkers were the most sensitive markers for predicting metabolic risks in the association obesity – diabetes –

hypertension. Obese women with type 2 diabetes and hypertension presented higher plasma glucose concentrations as compared to those of the control group. This probably reflects the status of inflammation and insulin resistance, which is in good agreement with the findings of previous studies [20-22]. Furthermore, high glucose contents can stimulate free radical production in obesity and lead to oxidative stress [23].

All obese women showed elevated plasma levels of *triglycerides and total cholesterol,* regardless of the associated pathology. Moreover, the highest concentrations were evident in obese women with diabetes and hypertension. *Overweight* and *obesity* are *linked* to lipid and lipoprotein metabolism abnormalities as well as to increased cardiovascular risk [20,21,24,25]. The results obtained provided evidence that plasma inflammatory biomarkers were high in obesity. It was confirmed that hypertension and type 2 diabetes related to obesity do aggravate the previously mentioned metabolic alterations. Multiple cytokines, such as TNF-α, IL-6, CRP and leptin, are produced by the adipose tissue. These cytokines are known to decrease insulin sensitivity and induce inflammatory processes, endothelial dysfunction, and atherosclerosis [11,24,26]. Our data suggest that leptin and IL-6 plasma levels were remarkably more important in obese women as compared to control subjects. Moreover, tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) levels were considerably more elevated in all obese groups as compared to their control counterparts. The highest values were observed in obese hypertensive diabetic and diabetic women. The results from this study reveal that all four markers under consideration are linked to obesity; they seem to be relevant and efficient inflammation biomarkers in obesity-associated diseases, like hypertension and diabetes. Several previous studies [2,3,11,26] have reported similar observations. A number of epidemiological studies indicated that there is a link between inflammation and metabolic diseases [1-3,11]. Circulating leptin levels are related to adipose tissue mass and inflammation. The elevated quantities of leptin in obese subjects reveal a connection between increased metabolic inflammation and adipokine. IL-6 is the major cytokine regulating the hepatic CRP production; it is produced by the adipose tissue. Several investigations have indicated that obese subjects have elevated IL-6 and CRP levels [3,11,26,27]. In addition, excessive amounts of CRP and IL-6 are predictive of type 2 diabetes development [1,11,27]. High CRP levels in hypertension, isolated, or in combination with obesity and diabetes mellitus, have been previously reported [2]. Moreover, obesity and associated complications are related to elevated oxidative stress [8,9]. Assessing biomarkers of oxidant/antioxidant status could help to diagnose patients and give practitioners a more objective significance of the antioxidant therapies and their efficacy in treating obese patients with complications. The results obtained suggest that erythrocyte antioxidant enzyme activities decreased significantly in obese women; the lowest values were obtained in obesity-HTA-type 2 diabetes and obesity-type 2 diabetes combinations. In addition, obese patients showed lower GSH levels than controls. Other researchers [2,8-10] reported comparable results. It was also found that, under high oxidative conditions, antioxidant enzymes may be consumed or inactivated. Consequently, increased oxidative stress causes reduction in antioxidants. Decreased plasma levels of vitamins C and E in all obese subjects could reflect their significant utilization rate in order to preclude the harmful action of free radicals in

obese patients, regardless of the related pathology. As a matter of choice, probably the decreased levels of vitamins C and E, which is certainly due to poorer dietetic intakes of these vitamins in obese women, contribute to weaker antioxidant defenses.

The decreased vitamin content was proved to be related to higher oxidative stress markers, like nitric oxide, superoxide anion, peroxynitrite, hydroperoxides, carbonyl proteins and conjugated dienes, mainly in obese diabetic and hypertensive diabetic women. Insulin resistance status, hypercholesterolemia, abnormal metabolism and metabolites in adipose tissue and/or exaggerated proinflammatory and inflammatory cytokines production could lead to higher amounts of oxidant markers in obese subjects [10]. The present study showed that erythrocyte O2°-, peroxynitrite and °NO contents were high in obese patients, particularly in those having diabetes with hypertension and type 2 diabetes, which is consistent with many other studies [6,10,28]. Many researchers have also provided evidence that obesity, with or without another disease, causes mitochondrial dysfunction with higher levels of mitochondrial reactive oxygen and nitrogen species. Greater amounts of erythrocyte carbonyl proteins were found in all obese patients, and especially in those presenting complications such as hypertension or type 2 diabetes, which is in good agreement with prior studies [2,9,25,28]. Carbonyl protein groups are adequate biomarkers of tissue damage that is engendered by high oxidative stress levels in obese patients having other complications. It is worth knowing that the amount of hydroperoxides was high in erythrocytes of obese women, suggesting that there is lipid peroxidation and intracellular oxidative stress, which is consistent with the findings of many researchers [2,9,28]. Obesity linked or not to diabetes and hypertension induced a remarkable growth in the oxidation rate and in conjugated dienes. One may assume that low density lipoproteins (LDLs) in obese patients are less resistant to oxidation in vitro as compared to control subjects [9,29], because LDL is sensitive to oxidation phenomena. Moreover, lower plasma levels of vitamins C and E, which are known to preclude LDL oxidation, were found in obese women [9,25].

The correlations found in this study indicate that markers of inflammation and biomarkers of oxidative stress are closely related. These correlations were particularly strong in obese women having hypertension and type 2 diabetes. In control women, leptin, IL-6, TNF-α and CRP were positively associated with all oxidative biomarkers (catalase, SOD, vitamin C, E, GSH, °NO, O2°-, peroxynitrite, carbonyl proteins, hydropeoxides, conjugated dienes, oxidation rate). These correlations showed that leptin, IL-6, CRP and TNF-α affected oxidant and antioxidant biomarkers in the same manner; the redox balance was then not changed in control subjects. In the current study, an increase in inflammatory markers in control non obese women induced an increase in oxidative damage with a concomitant increase in antioxidant status, indicating oxidative balance. This suggests that moderate inflammation in the absence of obesity or other pathology results in the upregulation of antioxidant defenses, providing a balance between the ROS-induced damage and the antioxidant systems.

Obesity alters these correlations that were abnormal compared to controls. In obese women without complications, leptin, IL-6, CRP and TNF-α were correlated positively with oxidant biomarkers (°NO, O2°-, peroxynitrite, carbonyl proteins, hydroperoxides, conjugated dienes, oxidation rate) and negatively with antioxidant biomarkers (catalase, SOD, vitamin C, E, GSH). These correlations were found to be stronger in

the presence of diabetes mellitus and hypertension, indicating an aggravation of inflammation and oxidative stress with obesity-related complications. Leptin, IL-6, TNF-α, and CRP have all been shown to be elevated in metabolic syndrome, and generally are correlated with adiposity and high oxidative stress [10,30,31]. Elevations in IL- 6, TNF-α, CRP and leptin levels were detected among obese T2DM subjects showing a strong direct relationship with oxidant markers and a strong inverse relationship with antioxidant markers [10]. The correlations between inflammatory markers and oxidative stress makers are well documented in obesity, diabetes and hypertension [9,10,31]. In our study, inflammation and oxidative stress stand out as a determinant process in the development of hypertension and diabetes in obese women.

From multiple regression analysis, BMI was a predictor of all inflammatory and oxidative stress biomarkers in women. The association BMI with complications constituted a risk factor and contributed to the aggravation of metabolic alterations. Associations between BMI, proinflammatory and oxidative biomarkers have been previously documented [30,32,33]. The clear relationship observed between inflammatory and oxidative marker concentrations and BMI shows the role of adipose tissue in initiating inflammation and oxidative stress [31]. In our study, the combination of obesity with hypertension and diabetes explained 27% to 53% of the *variation of vitamin C, vitamin E, GSH*, °NO, carbonyl proteins, hydroperoxides, conjugated dienes, oxidation rate, IL-6 and TNF-α. These results suggest the potential involvement of other factors in the variations of these inflammatory and oxidative stress biomarkers in women. However, the interaction BMI – complications explained 77% to 83% of the variation of catalase, SOD, O2°-, peroxynitrite, leptin and CRP. Our findings suggest that these individual oxidative stress and inflammatory biomarkers may provide valuable information when obesity, hypertension and diabetes are co-presenting.

Conclusions

Obese women with type 2 diabetes and hypertension had a higher inflammation status and a higher level of oxidative stress. There was a significant positive correlation between inflammation status and oxidative stress especially when obesity was associated to complications. Leptin and CRP were the best inflammatory biomarkers while catalase, SOD, O2°-, peroxynitrite were the best oxidative stress biomarkers that can predict diabetes and hypertension in obese women. Strategies to lower inflammation and oxidative stress including weight loss, physical activity and antioxidant-rich diet could represent an ideal therapy in obese women reversing complication risks.

Acknowledgments. This work was supported by the Algerian Research Project (PNR) from the Algerian Health investigation office (ATRSS). Our thanks go to all volunteers. The authors report that they have no conflicts of interest.

Disclosure. None declared.

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