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Relationship between Oxidized-LDL and Resistin Levels in Obese Diabetic Subjects

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ABSTRACT

This clinical study aimed to estimate Oxidized LDL and resistin levels in obese diabetic patients and to study their relationship with other biochemical and anthropometric variables. We hypothesized an in vivo relationship between Ox-LDL and resistin levels. This study was performed in the National Institute of Nutrition and Food Technology of Tunisia between January 2012 and July 2012 and included 26 obese diabetic patients and 21 healthy controls. Serum glucose, total cholesterol (TC), HDL-Cholesterol (HDL-C), triglyceride (TG) were measured by an enzymatic colorimetric method. Insulin levels were measured by chemiluminescence immunoassay method. Insulinresistance was assessed by HOMA-IR. Oxidized LDL and resistin levels were measured by Enzyme-linked Immunosorbent-Assay**.** Ox-LDL showed a positive correlation with resistin ($r=0.822$, $p<0.0001$), a significant association was also found between Ox-LDL and body mass index ($r=0.728$, $p<0.0001$) and LDL-C ($r=0.855$, $p<0.0001$). After multivariate regression analysis only LDL-C and BMI remained associated to Ox-LDL. The relationship between Ox-LDL and resistin was suppressed. In conclusion, Resistin could have a role in the onset of atherosclerosis but it's not a predictor.

KEYWORDS: Resistin, Oxidized-LDL, atherosclerosis, diabesity, obesity, Type 2 Diabetes Mellitus, Lipid oxidation.

1. INTRODUCTION

Obesity and Type 2 Diabetes Mellitus, that is, diabesity, are associated with an increased risk of cardiovascular complications, for example, atherosclerosis and myocardial infarction [1]. Recent studies have shown that numbers of bioactive molecules, known as adipokines, secreted from adipose tissue contribute this connection. Resistin, one of these adipokines, is a recently discovered fat-specific hormone that links obesity to insulin resistance [2]. Interestingly, resistin in humans is known to be expressed in atherosclerotic plaque [3]. And its plasma concentrations are increased in obesity and diabetes [4]. Resistin acts as an inflammatory cytokine and may play a critical role in atherosclerosis and other inflammatory diseases [3]**.** A recent study showed that resistin aggravates atherosclerosis by stimulating monocytes, endothelial cells, and vascular smooth muscle cells to induce vascular inflammation [5].

During atherosclerosis, LDL oxidation is an important step in the formation of plaque; in fact, the presence of macrophages in the vascular wall seems to be related to the presence of oxidized LDL. LDL oxidation takes place in situ in the intimal espace and involves different mechanisms [23].

Numerous studies have shown that the oxidation of low-density lipoprotein (oxLDL) was a useful marker for cardiovascular disease [6-7]. The increased oxidative stress, reflected by elevated levels of Oxidized LDL and risk factors are known to enhance atherosclerosis, damages the endothelium of the arterial wall [8]. The elevation of ox-LDL levels in atherosclerotic plaques is an important event in the development of atherosclerosis [9]. Several risk factors of atherosclerosis have been also studied with respect to their possible effects on LDL oxidation [10].

In light of these findings, we estimated circulating oxidized low-density lipoprotein (oxLDL) levels in obese diabetic patients and we hypothesized an in vivo relationship with resistin.

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METHODS

This clinical Trial was performed at the National Institute of Nutrition and Food Technology of Tunisia. Between January 2012 and July 2012.Twenty-seven (26) obese diabetic patients were recruited, with no history of vascular disease, with normal ECG findings at exercice and normal peripheral artery dopler ultrasonography findings. Patients in this group were treated by oral hypoglycemic agents, mainly glibenclamide and metformin. Type 2 diabetes patients taking insulin were excluded from this study. The exclusion criteria were the presence of sustained type 1 diabetes, acute and chronic infections, malignancy, hepatic or renal disease, diabetic retinopathy, and other endocrine dysfunctions. The control group consisted of Twenty-one (21) with no history of endocrine disorders, hypertension, or coronary heart diseases, non obese, non-diabetic according to an oral glucose tolerance test and healthy according to a physical examination and routine laboratory test. None of the subjects had received any medication (hormone therapy, corticosteroids, antioxidant formulations and thiazolidinediones) which may have affected insulin resistance and/or endothelial function and none of the subjects were current smokers and consumers of alcohol. The control group was recruited from the population listed on the local register of the Tunis city and consisted of patients who came for a routine checkup. Both patients and controls belonged to the same ethnic background and all shared a common geographic origin in North Tunisia. The research protocol was approved by the local ethics committee and informed written consent was obtained from each subjects. Type 2 diabetes was defined as a fasting blood glucose level >7.0 mmol/L, the use of anti-diabetic drugs, or both. Weight and height were measured on the subjects barefooted and lightly clothed.

After a 12 h overnight fast, samples were drawn from the patients and controls between 7.00 and 9.00 a.m. via the venipuncture of an antecubital vein. Blood samples were collected in vacuntainer tubes with a gel separator and in heparinized tubes and were centrifuged at 2000 rpm for 15 min at 4°C. All biochemical variables were measured on the same day of the blood collection and a 1-ml aliquot of plasma was rapidly frozen (-20°C) for subsequent blood biochemical analysis, resistin and Ox-LDL levels.

Serum glucose, total cholesterol (TC), HDL-Cholesterol (HDL-C), triglyceride (TG) were measured by an enzymatic colorimetric method. Insulin levels were measured by chemiluminescence immunoassay method. The concentration of Ox-LDL in plasma was measured with a sandwich Enzyme-Linked Immunosorbent Assay procedure using a peroxidase-conjugated antibody and a Tetramethylbenzidine (TMB) as a substrate for peroxidase with a sensitivity of 4.130 ng/ml (ref K7810, Immundiagnostic, Bensheim). Resistin levels were measured using an ELISA (CAT. #EZHR-95K Millipore (6 Research Park Drive, St. Charles, Missouri 63304 USA)) with sensitivity 0.16 ng/mL, with Inter-assay 7.1–7.7% and Intra-assay: 3.2–7.0%. The fasting plasma glucose was determined with the glucose oxydase enzymatic method (Beckman synchron Cx9).

Low density lipoprotein-cholesterol (LDL-C) was calculated using the Friedwald formula. Standing height and body weight were measured with the subjects dressed in light indoor clothing barefooted. The BMI was calculated as body weight (kg) divided by the square of the height (m^2). Obesity was defined as BMI≥30 kg/m2 as defined by the World Health Organisation World Health Organisation. Physical status: the use and interpretation anthropometry. Insulin resistance (IR) was assessed using the Homeostasis model assessment (HOMA-IR), according to the following formula: HOMA-IR= fasting insulin concentrations (µUI /mL)*fasting glucose concentrations (mmol/L) /22.5, LDL-cholesterol was computed by the Friedwald formula.

Statistical analysis: All statistical analyses were conducted using the analysis software SPSS 11.5 (SPSS.Inc, Chicago, USA) statistical package. For all tests performed, a p value of less than 0.05 was considered as significant. Clinical and biochemical features of the population are presented as mean±S.D. Pearson correlation coefficients were calculated to quantify the univariate associations among variables. Multiple linear regression analysis was performed to examine the multivariate correlations of Ox-LDL with all variables.

RESULTS

The study group, whose characteristics are shown in Table 1, consisted of 26 Obese diabetic patients (age=38.85±13.39) and 21 healthy controls (age=37.81±8.95). At the time of assessment, all of the study subjects were free of history of cardiovascular events (e.i. ischemic heart disease, cerebrovascular disease) and had no clinical evidence of atherosclerotic diseases. The comparison of the clinical variables between healthy controls and obese diabetic groups are shown in Table 1. There were no significant difference between healthy controls and obese diabetic groups for age, TC, TG and HDL-C Compared to healthy controls, obese diabetic patients had significantly higher BMI, LDL, fasting blood glucose, insulin and HOMA-IR. Oxidized-LDL and resistin levels were significantly higher in obese diabetic patients compared to healthy controls ($p<0.01$).

In order to study the correlations between all the measured parameters, we used the univariate analysis as shown in table 2. We found that resistin was significantly and positively correlation with BMI, Insulin and HOMA-IR.

Table 3 represents the univariate correlation coefficients and linear regression analysis of the relationship between Ox-LDL and biochemical parameters. In univariate analysis, Oxidized LDL was significantly, positively and strongly correlated with BMI (r=0.728, p<0.01), insulin (r=0.631, p<0.01), resistin (r=0.822, p<0.01) and LDL cholesterol (r=0.855, p<0.01). Oxidized LDL was not significantly associated neither to HOMA-IR in obese diabetic patients (r=0.259, p=0.201) nor to Fasting blood glucose (r=0.250, p=0.218). Variables that on univariate analysis were found to be correlated with Ox-LDL were used in further analysis. A multivariate linear regression analysis with Ox-LDL as dependent variable was performed including parameters that were univariately correlated with Ox-LDL with P-value<0.01. LDL-C and BMI remained significantly correlated with Ox-LDL levels.

Table 1: Anthropometric and biological characteristics of the study population

	Healthy Controls(n=21)	Obese diabetic patients (n=26)	P-value
Age (years)	37.81±8.95	38.85±13.39	0.983
BMI (kg/m²)	19.95±2.17	35.43±4.34	0.000
TC (mmol/l)	4.52±1.02	5.03±1.17	0.375
TG (mmol/l)	1.39±0.95	2.18±1.12	Not significant
HDL-C (mmol/l)	1.05±0.22	1.00±0.24	Not significant
LDL-C (mmol/m)	2.22±0.27	3.73±0.72	0.000
FBG (mmol/l)	5.04±0.57	10.18±1.31	0.000
Insulin (UI/ml)	3.11±2.00	15.23±3.46	0.000
Ox-LDL	56.32±25.00	321.05±78.60	0.000
HOMA-IR	0.88±0.59	6.01±2.02	0.000
Resistin	1.61±1.51	11.16±2.40	0.000

Values are expressed as mean±SD. BMI, Body Mass Index; TC, Total cholesterol; TG, triglycerides; HDL-C:; high density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, Fasting Blood glucose; Ox-LDL, Oxidized-LDL, HOMA-IR, Homeostatic Model Assessment index -Insulin Resistance. p-value is significant at 0.05 (p<0.05).

Table 2: Relationship between resistin and biochemical and anthropometric features

	Resistin	
	Healthy controls (n=21)	Diabetic obese (n=26)
FBG	0.151	0.326
TC	-0.026	-0.075
TG	0.124	-0.013
HDL-C	-0.274	0.020
BMI	0.591[b]	0.474[c]
Insulin	0.342	0.468[c]
HOMA-IR	0.437[c]	0.683[b]
Ox-LDL	0.406	0.822[b]

[a]Values shown are correlation coefficients (r). FBG, Fasting Blood glucose ; TC, Total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein-cholesterol; BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment index -Insulin Resistance; Ox-LDL, Oxidized-LDL. [b]significant at p<0.01, [c] significant at p<0.05.

Table 3: Univariate correlation coefficients and linear regression analysis of the relationship between Ox-LDL and biochemical parameters in the sample of 26 obese diabetic subjects

	Univariate analysis		Multivariate analysis		
	Correlation		Regression[a]		
	r	p-value	b	S.E.[b]	p-value
LDL-C	0.855	0.000	67.286	11.118	0.000
BMI	0.728	0.000	5.945	1.381	0.000
FBG	0.250	0.218	-	-	-
resistin	0.822	0.000	-4.033	5.471	0.470
Insulin	0.631	0.001	-0.966	2.536	0.708

FBG, Fasting Blood glucose; BMI, Body Mass Index; LDL-C, low density lipoprotein-cholesterol. [a]The regression model includes the variables: LDL-C, BMI, fasting blood glucose, resistin and insulin. [b]S.E. means standard error of the b coefficients. p-value is significant at 0.05 (p<0.05).

DISCUSSION

Much attention has been focused on the potential role of adipose tissue in the development of vascular complications of both obesity and diabetes. This study was aimed to estimate levels of resistin and circulating oxidized low-density lipoprotein (oxLDL) levels in obese diabetic patients and evaluate their in vivo relationship. Resistin is a protein secreted by adipocytes and leads to insulin resistance, suggesting a possible link between obesity and diabetes [11]. In the present study we found higher plasma resistin levels in obese

diabetic patients compared to controls. Mean plasma resistin levels were correlated with BMI and insulin resistance as assessed by HOMA-IR. Mojiminiyi *et al* found that resistin is positively associated with markers of obesity, inflammation and insulin resistance but negatively correlated with markers of insulin sensitivity. Furthermore, these associations were BMI-dependent associations suggesting that Resistin may represent a link between obesity and insulin resistance via pro-inflammatory pathways [12].

Oxidative modification of lipids is a common part of inflammatory diseases including diabetes and obesity. Recently, Holvoet P et al., have measured circulating levels of Ox-LDL in patients with cardiovascular disease supporting their possible role as a marker of asymptomatic cardiovascular disease [11] Oxidized LDL was suggested to play a crucial role in atherogenesis, and both diabetes and obesity are associated with atherosclerotic complications [13]. We investigated the levels of Oxidized LDL in obese diabetic patients without clinical evidence of cardiovascular disease. In this setting, we found a significant increase level of circulating plasma Ox-LDL in agreement with previous reports [14]. Another study, showed that ox-LDL level increases with the length of diabetes, even though the patients' LDL-cholesterol level is maintained at a desirable level [14]

In the present study, univariate analysis revealed that plasma levels of oxLDL correlated with body mass index (BMI). Keaney et al. [15], recently, reported that obesity, measured by BMI, is independently associated with oxidative stress in the Framingham Heart Study.

Few studies have investigated the relationship between Ox-LDL and adipokines, but none have focused on their relationship to resistin. We hypothesized a possible association between resistin to Oxidized LDL in vivo. Our results demonstrate that concentrations of Ox-LDL and resistin were significantly related at univariate analysis ($r=0.822$, $p<0.01$) but this association was suppressed after multivariate analysis including all metabolic and anthropometric variables univariably correlated, this relationship was a novel result. Thus, the mechanism of the link between resistin and Ox-LDL is not clear. However, resistin, has been found to possess proinflammatory properties in humans and emerged as a promising inflammatory marker [16], it was suggested to aggravates atherosclerosis by stimulating monocytes, endothelial cells, and vascular smooth muscle cells to induce vascular inflammation [6]. These findings provide the first insight on the causal relationship between resistin and atherosclerosis. [17]. Further, resistin has been noted to play a vital role in increasing the level of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in an obese person [18] which is directly atherogenic. However, the function of resistin in humans is enigmatic. Recently, Melone et al. revealed for the first time that resistin is a highly attractive therapeutic target in ameliorating elevated serum low-density lipoprotein and, thereby, atherosclerotic cardiovascular diseases in obese humans [19]. Our data suggests that resistin could have a role in the onset of cardiovascular disease but it’s not directly related to oxidized-LDL.

Multivariate analysis revealed that BMI is the strongest predictor of circulating oxLDL levels. In this setting, our data agree with previous reports [10] supporting a possible link between body fat mass and LDL oxidation. Our data showed also a strong association between Ox-LDL and LDL-cholesterol which is a strong predictor of circulating Ox-LDL, this finding suggests that dyslipidemia particularly promotes the oxidation of LDL in the subendothelium [20]. In addition, studies that have investigated the reduction of total body fat have shown reductions in plasma markers of oxidative stress after weight loss induced by diet [21]. Furthermore, a population-based study has shown significant correlation of ox-LDL/LDL ratio [22] with BMI. These findings indicated that weight is an important determinant for oxidative stress. In conclusion, the present study shows a new correlation between Ox-LDL concentrations and resistin levels in obese diabetic patients, however resistin seems to be not directly related to oxidized-LDL, further studies are necessary to better explain this relationship.

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Conflict of interest: None.

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