Relationship between Plasma Antioxidant Status and Leptin in Controlled and Non-Controlled Type 2 Diabetic Non-Obese Women

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It is an established fact that diabetes induces oxidative stress; obesity is associated with type 2 diabetes mellitus (T2DM) and increased leptin levels. Insulin has been suggested to be a regulator of in vivo leptin secretion, while hyperinsulinaemia is a feature of T2DM. Our study aimed at determining the relationship between plasma antioxidant status and leptin in controlled and non-controlled T2DM non-obese women.

**Materials and Methods:** Sixty-five non-obese (BMI <26kg/m2) women with T2DM, 34 controlled (HbA1c <6%) and 31 non-controlled (HbA1c >8%), between the ages of 25-55 years were recruited for the study. Plasma levels of leptin, α-tocopherol, retinol, total antioxidant status (TAS), lipid peroxidation [Malondialdehyde(MDA)], fasting plasma glucose(FPG), glycated haemoglobin (HbA1c %), total cholesterol(TC), HDL-cholesterol, LDL-cholesterol and triglyceride (TG) were determined for all enrollees. **Results:** Mean±SD plasma α-tocopherol and TAS for non-controlled T2DM subjects were significantly reduced compared to the controlled group (p<0.01). The analysis for association between leptin and TAS shows an inverse correlation for the controlled (r=-0.23, p<0.05) and for the non-controlled (r=-0.51, p<0.01) T2DM group. Likewise, there was an inverse correlation between leptin and α-tocopherol for the controlled (r=-0.25, p<0.05) and for the non-controlled (r=-0.49, p<0.01) T2DM groups. However, a direct correlation between leptin and MDA was found for the controlled (r=0.21, p<0.05) and for the non-controlled (r=0.47, p<0.01) T2DM subjects. **Conclusion:** Our findings suggest that oxidative stress and leptin are associated with risk of T2DM and could be a target for insulin sensitization to prevent diabetes and its complications.

**Key Words:** Leptin, Oxidative stress markers, Type 2 diabetes mellitus

**Received:** 21.10.2009 **Accepted:** 10.06.2010

**Introduction**

Diabetes mellitus is a heterogeneous condition reflecting different metabolic disorders accompanied by a variety of complications. Worldwide over 90% of patients with diabetes are those with T2DM, which is characterized by insulin resistance and relative rather than absolute insulin deficiency.
Evans et al. reported that oxidative stress leads to tissue damage and has been linked to the impairment of insulin action and β-cell function, with the resultant development of T2DM\textsuperscript{3}. Oxidative stress can result in widespread lipid, protein and DNA damage, including oxidative modification of LDL cholesterol, believed to be central in the pathogenesis of atherosclerosis and endothelial dysfunction\textsuperscript{4}. Oxygen-derived free radicals have been implicated in the pathophysiology of various disease states, including diabetes mellitus\textsuperscript{5}. Diabetes mellitus is also characterized by increased generation of glycoxidation products associated with the advanced oxidative stress\textsuperscript{6}.

The relationship between hyperinsulinemia and free radical production was revealed in exposure of intact human fat cells to insulin and leads to a time- and dose-dependent accumulation of hydrogen peroxide in the suspension medium\textsuperscript{7}. In addition, increased insulin concentration in animals following intraperitoneal injection of dextrose has been found to be associated with increased free radical production\textsuperscript{8}.

Leptin, a 16-kDa hormone identified and cloned in 1994, is synthesized and secreted specifically from white adipose cells\textsuperscript{9}. A recent study has demonstrated that adipose tissue is an active endocrine tissue, which secretes hormones such as leptin, tumour necrosis factor-α, plasminogen activator inhibitor-1, adiponectin, resistin and interleukin-6, referred to as adipocytokines\textsuperscript{10}. Recent studies have shown that leptin has peripheral actions to stimulate vascular inflammation, oxidative stress and vascular smooth muscle hypertrophy that may contribute to pathogenesis of T2DM, hypertension, atherosclerosis and coronary heart disease\textsuperscript{11-13}. Leptin plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditures\textsuperscript{14}. Leptin resistance is related to the development of insulin resistance in individuals with T2DM\textsuperscript{15}. The development of T2DM in association with obesity, hyperinsulinemia and insulin resistance has been demonstrated and obesity is associated with a marked increase in circulating leptin concentration\textsuperscript{16}. Stefanović et al. observed a positive correlation between lipid peroxidation and leptin in obese patients, which suggests that increased oxidative stress and hyperleptinemia, both consequences of obesity, may play a role in T2DM development\textsuperscript{17}. In an animal study, it was reported that leptin increases formation of reactive oxygen species (ROS) in a process coupled with increased fatty acid oxidation and activation of protein kinase A in endothelial cells\textsuperscript{18}. One school of thought postulated that fat soluble antioxidant vitamins may furthermore play a role in the preservation of insulin action through the maintenance of endothelial function\textsuperscript{19}. Endothelial dysfunction has recently been linked to abnormal glucose homeostasis\textsuperscript{20}. Schmidt et al. reported that high leptin levels, probably reflecting leptin resistance, predict an increased risk of diabetes; adjusting for factors purportedly related to leptin resistance unveils a protective association, independent of adiponectin and consistent with some of leptin’s described protective effects against diabetes\textsuperscript{21}. These reports make the issue of leptin and diabetes a matter of controversy. However, there is a dearth of information regarding the relationship between plasma concentrations of antioxidant status and leptin in T2DM. The aim of this study is to determine the relationship between plasma antioxidant status and leptin in controlled and non-controlled T2DM non obese women.

**Materials and Methods:**

The case-control study was conducted on 65 T2DM Nigerian women between the ages of 25-55 years, attending the medical outpatient department of the Lagos General Hospital, Lagos in South Western Nigeria. After the approval of the Ethic committee of the hospital, the recruited T2DM women were screened by medical history, physical examination, fasting blood glucose and BMI.
< 26 kg/m², and divided into two groups based on their glycosylated haemoglobin (HbA1c) percentage. Group A comprised 34 controlled (HbA1c <6%) and group B had 31 non-controlled (HbA1c >8%)22. Based on the patients medical records, the duration of the T2DM was between 60-96 months, none of them was on insulin therapy or had any complications. After all subjects gave informed consent, clinical parameters were taken; none were on oral vitamins two weeks prior to or during the study period. They were instructed not to take any fruit or juices but to take only plain water during the study period and to report any abnormality observed before the study.

Diabetes mellitus was diagnosed according to WHO criteria and the subjects were classified as T2DM using WHO criteria23. After an overnight fasting for 10-12 hours, 10mL of venous blood was collected from each patient, 5mL into a lithium heparin bottle, 2mL into a fluoride oxalate bottle and 3mL into an EDTA bottle. The respective supernatants, obtained after centrifugation at 2500 rpm for 10 minutes, were frozen at -20°C. Total cholesterol (TC), HDL-cholesterol, TG and FPG were analysed using the enzymatic cholesterol esterase method, direct enzymatic, pyruvate kinase method and the glucose oxidase method on the Beckman synchrone CX5 auto analyzer respectively. LDL-cholesterol was calculated using the Friedwald formula (LDL cholesterol = total cholesterol – (triglycerides / 5 + HDL cholesterol).

Malondialdehyde (MDA) a secondary product of lipid peroxidation was measured according to the Satoh method24. Measurement of total antioxidant status (TAS) in the plasma was performed using a commercial kit from Randox Laboratories (Randox Laboratories Ltd, Diamond Road, Crumlin, Co. Antrim, Ireland)25. The assay was calibrated using 6-hydroxy-2, 5, 8-tetramethylchroman-2-carboxylic acid (trolox). The results were expressed as mmol/L of trolox equivalent. Plasma Leptin concentrations was measured by an enzymatic amplified ‘two-step’ sandwich type immunoassay, using the commercially available cat #1742-6 Human leptin Enzyme-linked immnosorbent ELISA Kit manufacture by Diagnostic Autometa Inc, Kansas, USA and supplied by Phillab Nig. Limited Lagos. Retinol and α-tocopherol were measured by high performance liquid chromatography. Whole blood samples for (HbA1c) were collected on EDTA and measured using the antigen antibody binding technique. The degree of agglutination is proportional to the amount of HbA1c adsorbed on to the surface of the latex particles. The amount of agglutination was then measured as absorbance and the respective HbA1c valued is obtained from a standardized calibration curve. The inter- and intra-assay coefficients of variation (CV), were 3.3% and 2.4% for leptin (at 5.4 ng/mL), 4.7% and 3.9% for glucose (at 4.5 mmol/L), 5.0% and 4.1% for HbA1c % (at 5.0%), 1.5% and 1.3% for triglycerides (at 1.3 mmol/L), 3.2% and 2.7% for total cholesterol (at 3.5 mmol/L), 1.1.0% and 1.8% for HDL-cholesterol (at 0.9mmol/L), 7 % and 5.6% for retinol (at 1.0 µmol/L), 5.1% and 2.6% for α-Tocopherol (at 5.5 µmol/L), 6.0% and 7.7% for TAS (at 3.5 mmol/ trolox Eq), and 7.9% and 5.6% for MDA (at 3.0 mmol/ mL), respectively.

Statistical analysis was done using the SPSS Software version 10.0 (SPSS, Chicago, IL, U.S.A.). Data are presented as mean±SD. Differences between the two groups were assessed by Student's t-test. Significance of the correlations was assessed by using Pearson's rank correlation analysis. Results were considered significant with p values of <0.05.

Results
A total of 65 non obese women with T2DM, 34 controlled (HbA1c < 6%), and 31 non-controlled (HbA1c >8%) participated in the study. Clinical and biochemical parameters are presented in tables 1 and 2 respectively. There was no significant difference between the mean ± SD of BMI.
Table 1. The clinical parameters (mean±SD) of the controlled T2DM and non-controlled T2DM patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T2DM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Controlled (n=34)</td>
</tr>
<tr>
<td>Duration of DM (months)</td>
<td>58.1±3.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.3±1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.7±2.3</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.6±2.1</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>121.9±2.5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.8±1.7</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>70.1±3.2</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>89.1±2.4</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.79±0.11</td>
</tr>
</tbody>
</table>

Table 2. The biochemical parameters (mean±SD) of the controlled T2DM and non-controlled T2DM patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controlled (n=34)</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.11±0.89</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.72±0.19</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.39±0.10</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.10±0.60</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.28±0.21</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.03±0.41</td>
</tr>
<tr>
<td>Retinol (µmol/L)</td>
<td>2.92±0.84</td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>23.25±1.90</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>8.77±1.80</td>
</tr>
<tr>
<td>TAS (mmol/L trolox Equivalent)</td>
<td>1.98±0.49</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>2.89±0.47</td>
</tr>
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</table>

The mean±SD of circulating plasma α-Tocopherol for non-controlled T2DM subjects (12.79±2.50 µmol/L) was significantly reduced compared to (23.25±1.90 µmol/L) for the controlled (p<0.01). However, the plasma leptin for the non-controlled T2DM...
subjects (10.94 ± 1.40ng/mL) was significantly increased compared to (8.77±1.80 ng/mL) for the controlled p<0.01. The mean± SD plasma concentration of TAS (0.79±0.07mmol/trolox Eq.) for non-controlled T2DM subjects was significantly reduced compared to TAS (1.98 ± 0.49 mmol/ trolox Eq.) for the controlled p<0.01. However, the mean± SD plasma concentration of MDA (4.36 ± 0.25nmol/ mL) for non-controlled T2DM subjects was significantly increased, compared to (2.89± 0.47 nmol/ mL) for the controlled p<0.01.

Fig. 1. Correlation between plasma total antioxidant status (TAS) and leptin in the (a) controlled (r = -0.23, p<0.05) and (b) non-controlled (r = -0.51, p<0.01) T2DM patients.

In Fig. 1, the analysis for association between leptin and TAS showed an inverse correlation (r = -0.23, p<0.05) for the control-led and (r = -0.51, p<0.01) for the non-controlled T2DM. Likewise, figures 2 a and b show an inverse correlation between leptin and α-tocopherol (r = -0.25, p<0.05) for the control-led and (r = -0.49, p<0.01) for the non-controlled T2DM. However, figures 3a and b show a direct correlation between leptin and MDA (r = 0.21, p<0.05) for the controlled and (r = 0.47, p<0.01) for the non-controlled T2DM.

Fig. 2: Correlation between plasma α-tocopherol (vitamin E) and leptin in the a) controlled (r = -0.25, p<0.05) and b) non-controlled (r = -0.49, p<0.01) T2DM patients.
Discussion

Our study showed significant increases in plasma MDA and leptin level in non-controlled T2DM subjects, compared to the controlled T2DM subjects. However, the plasma level of TAS and circulating \(\alpha\)-tocopherol was significantly reduced in non-controlled T2DM subjects compared to the controlled T2DM ones. The plasma concentrations of TAS and \(\alpha\)-tocopherol showed a strong inverse correlation with leptin level in non-controlled T2DM individuals, which is an indication of marked oxidative stress. However, marker of lipid peroxidation MDA showed a strong direct correlation with leptin level in non-controlled T2DM subjects compared to the controlled. These findings point to the fact that individuals with diabetes are in state of oxidative stress. Van der Jagt et al., in their study, reported increased lipid peroxidation, which can be detected in the early stages of T2DM, well before the development of any diabetic complications. Several different mechanisms have been proposed to explain why oxidative stress is increased in diabetes mellitus; these mechanisms fall into two general categories: Increased production of ROS and decreased antioxidant defences. Hyperglycaemia in diabetes mellitus may increase ROS production via changes in the redox potential of glutathione and decreased antioxidant defences due to reduction in total antioxidant capacity in plasma. Some of these mechanisms may possibly operate simultaneously in a synergistic fashion. Increased HbA1c and decreased glycaemic control have been linked to both increased rate of lipid peroxidation and impaired antioxidant scavengers in subjects with diabetes, findings, which are inconsistent with ours. Our finding however is also in line with that of the study of Yamagishi et al. who reported that leptin increases oxygen-reactive species by promoting increased fatty acid oxidation.

Fat soluble antioxidant vitamin, \(\alpha\)-tocopherol was significantly reduced in uncontrolled T2DM patients, compared to the controlled subjects in this study, a finding similar to the finding of Sundarm et al., who reported low levels of \(\alpha\)-tocopherol and ascorbic acid in diabetic patients. In our study, the low levels of \(\alpha\)-tocopherol could reflect their high consumptive rate, due to mopping up of increased free radicals generated in T2DM. According to Therond et al., oxidative stress is induced by both increases in free radicals and disturbance of the free radical scavenging system in diabetes mellitus. Alternatively, it is also possible...
that reduced α-tocopherol concentrations reflect low dietary intake, which can also account for the decreased antioxidant defence system in diabetic subjects.

In the present study, we tried to avoid possible bias or confounders, by choosing a homogenous cohort made up of Nigerians with T2DM who were of the same sex and BMI <26 Kg/m² because of the influence of these factors on plasma leptin. We observed a significant increased plasma level of leptin in non-controlled T2DM subjects compared to the controlled T2DM, in this study. Our findings corroborate the finding of Wu et al. who demonstrated that leptin levels in diabetics are higher than in normal subjects and that T2DM is associated with hyperinsulinaemia and insulin resistance. Segal et al. reported that basal plasma leptin concentrations was significantly higher in lean insulin-resistant than in lean insulin sensitive subjects, independent of body fat mass. This was established in our present study, as the plasma concentration of leptin was significantly increased in non-controlled T2DM patients, compared to the controlled, which is independent of BMI. In the non-controlled T2DM, plasma triglyceride concentrations were increased which may lead to expansion of the volume of fat cells in non-controlled T2DM individuals, which in turn may lead to an increase in ob gene expression and plasma leptin concentrations. It is also possible that plasma triglyceride concentrations are affected by leptin, through indirect mechanisms involved in insulin resistance. Overall, there is overwhelming evidence that leptin and antioxidant capacity are associated with T2DM, which may be targeted in the control of T2DM and the complications associated with it.

In conclusion the data indicate that systemic oxidative stress is associated with leptin in individuals with poor control of T2DM, based on the strong correlation between leptin and markers of oxidative stress in individuals with non-controlled T2DM. Oxidative stress and leptin are associated with risk of T2DM and could be a target for insulin sensitization to prevent diabetes and its complications. Further large-scale studies investigating the physiopathologic mechanisms are required to clarify the relationship between leptin, oxidative stress and diabetes.

Acknowledgments:
We are thankful to the staff of Department of Chemical Pathology, Lagos State Laboratory Services, General Hospital, Lagos Nigeria for their assistance during the research period.

References


