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

Ajala MO[a], Ogunro PS[b], Idogun SE[c], Osundeko O[d]

[a]Department of Chemical Pathology, Lagos State Laboratory Services, General Hospital, Lagos Nigeria, [b]Department of Chemical Pathology, College of Health Science, Ladoke Akintola University of Technology, Osogbo, Nigeria, [c]Department of Chemical Pathology, College of Medicine, University of Benin, Benin City, Nigeria, [d]Department of Endocrinology, Pennsylvania District Hospital, Pennsylvania, USA

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), glycosylated 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 (p<0.01). However, the mean± SD plasma leptin and MDA for the non-controlled T2DM subjects were significantly increased 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.

Correspondence: P.S. Ogunro, Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria
E-mail: ogunrops@yahoo.com

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[1], which is characterized by insulin resistance and relative rather than absolute insulin deficiency[2].

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[3]. Oxidative stress can result in widespread lipid, protein and DNA damage, including oxidative modification of LDL choleserol, believed to be central in the pathogenesis of atherosclerosis and endthelial dysfunction[4]. Oxygen-derived free radicals have been implicated in the patophysiology of various disease states, incuding diabetes mellitus[5]. Diabetes mellitus is also characterized by increased generation of glycoxidation products associated with the advanced oxidative stress[6].

The relationship between hyperinslinemia 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 peroxdase in the suspension medium[7]. In addition, increased insulin concentration in animals following intraperitoneal injection of dextose has been found to be associated with increased free radical production[8].

Leptin, a 16-kDa hormone identified and cloned in 1994, is synthesized and secreted specifically from white adipose cells[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 inhiitor-I, adiponectin, resistin and interleukin-6, referred to as adipocytokines[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 pathoenesis of T2DM, hypertension, atheroslerosis and coronary heart disease[11-13]. Leptin plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditures[14]. Leptin resistance is related to the development of insulin resistance in individuals with T2DM[15]. The development of T2DM in association with obesity, hyperinsulinemia and insulin resis-

tance has been demonstrated and obesity is associated with a marked increase in circulating leptin concentration[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[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[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[19]. Endothelial dysfunction has recently been linked to abnormal glucose homeostasis[20]. Schmidit 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[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/m^2, 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 criteria[23]. 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 -20ºC. 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 method[24]. 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 mmo/L of trolox equivalent. Plasma Leptin concetrations was measured by an enzymatic amplified 'two-step' sandwich type immunoassay, using the commercially available cat #1742-6 Human leptin Enzyme-linked immnsorbent ELISA Kit manufacture by Diagnotic 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 nmol/ 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

and or the clinical parameters for the controlled, when compared to the non- controlled T2DM subjects.

Table 1. The clinical parameters (mean±SD) of the controlled T2DM and non-controlled T2DM patients

Parameters	T2DM	
	Controlled (n=34)	Non-controlled (n=31)
Duration of DM(months)	58.1±3.7	60.1±5.4
Height (cm)	160.3±1.6	158.9±2.1
Weight (kg)	60.7± 2.3	59.8±3.1
BMI (Kg/m2)	23.6±2.1	23.7±1.9
Systolic BP (mmHg)	121.9±2.5	130.2±3.8
Diastolic BP (mmHg)	79.8±1.7	85.3±2.1
Waist Circumference (cm)	70.1±3.2	69.7±2.1
Hip Circumference (cm)	89.1±2.4	90.1±1.9
W/H ratio	0.79±0.11	0.77±0.08

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

Parameters	T2DM		p value
	Controlled (n=34)	Non-controlled (n=31)	
FPG(mmol/L)	5.11±0.89	8.55±1.80	0.034
HbA1c (%)	5.72±0.19	8.80±0.22	0.032
Triglyceride(mmol/L)	1.39±0.10	2.40±0.18	0.021
Cholesterol (mmol/L)	4.10±0.60	5.91±0.31	0.081
HDL-cholesterol (mmol/L)	1.28±0.21	0.89±0.16	0.037
LDL-cholesterol (mmol/L)	2.03±0.41	3.82±0.32	0.036
Retinol (µmol/L)	2.92±0.84	2.26±0.40	0.127
α-Tocopherol (µmol/L)	23.25±1.90	12.79±2.50	0.007
Leptin (ng/mL)	8.77±1.80	10.94±1.40	0.031
TAS (mmol/L trolox Equivalent)	1.98±0.49	0.79±0.07	0.004
MDA (nmol/mL)	2.89±0.47	4.36±0.25	0.009

The mean±SD of circulating plasma α-Tocopherol for non-contolled 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.
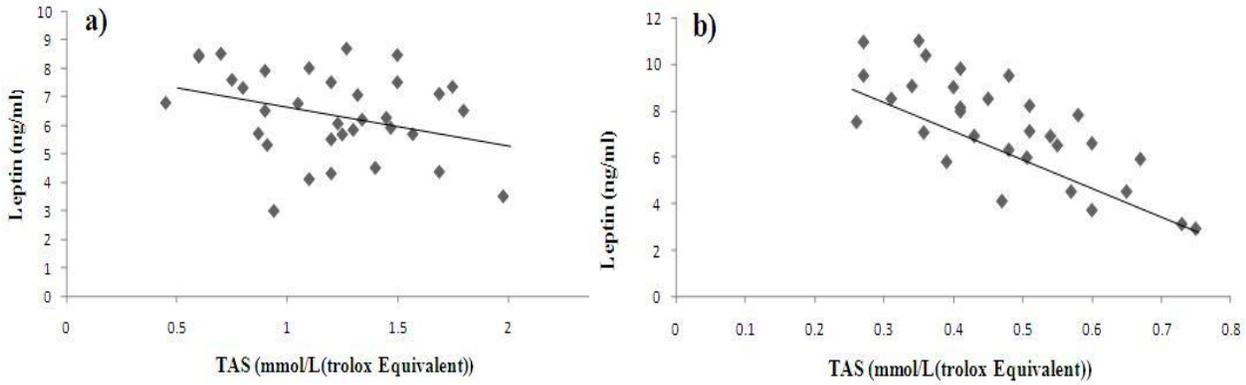

Fig. 1. Correlation between plasma total antioxidant status (TAS) and leptin in the (a) controlled (r = -0.23, p<0.05) and (b) non-controlled (r = -0.51, p<0.01) T2DM patients.

In Fig. 1, the analysis for association between leptin and TAS showed an inverse correlation (r = -0.23, p<0.05) for the controlled 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.
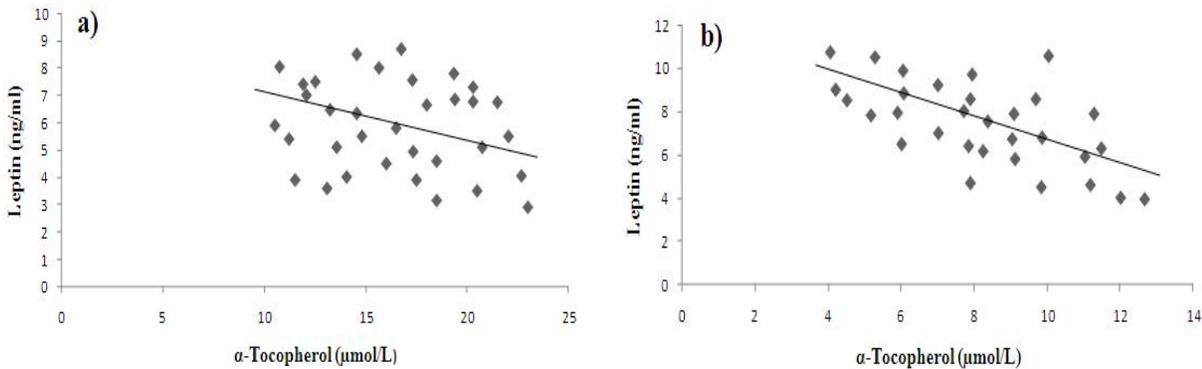

Fig. 2: Correlation between plasma α-tocopherol (vitamin E) and leptin in the a) controlled (r = -0.25, p<0.05) and b) non-controlled (r = -0.49, p<0.01) T2DM patients.
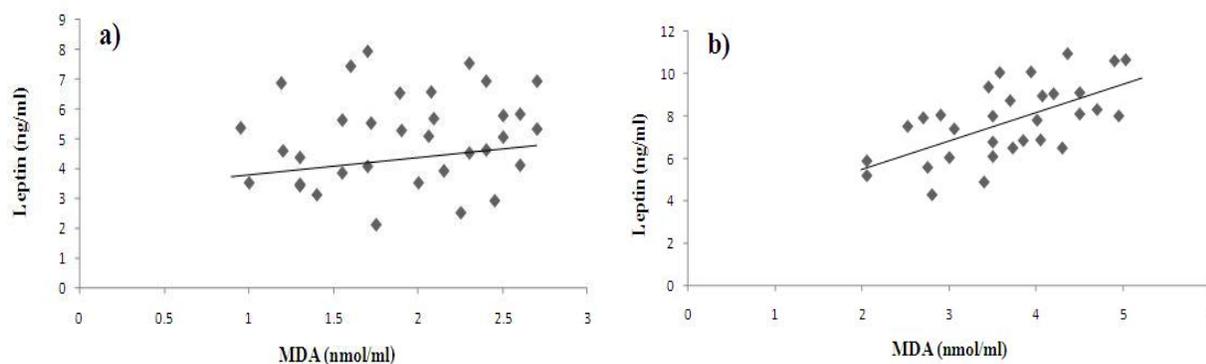
Fig. 3: Correlation between plasma malondialdehyde (MDA) and leptin in the a) controlled (r = 0.21, p<0.05) and b) non-controlled (r = 0.47, p <0.01) T2DM patients.

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 α-tocopherol was significantly reduced in non-controlled T2DM subjects compared to the controlled T2DM ones. The plasma concentrations of TAS and α-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 per oxidation, which can be detected in the early stages of T2DM, well before the development of any diabetic complications[26]. 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 produc-

ction via changes in the redox potential of glutathione[27] and decreased antioxidant defences due to reduction in total antioxidant capacity in plasma[28]. 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[27], 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[18].

Fat soluble antioxidant vitamin, α-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 α-tocopherol and ascorbic acid in diabetic patients[29]. In our study, the low levels of α-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[30]. Alternatively, it is also possible

that reduced α-tocopherol concentrations reflect low dietary intake, which can also account for the decreased antixiant 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/m^2 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[31]. 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[32]. 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[33]. 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 it complications. Further large-scale studies investigating the physio-pathologic mechanisms are required to clarify the relationship between leptin, oxidative stress and diabetes.

References

1. Dunstan DW, Zimmet PZ, Welborn TA, Shaw J, de Courten M, Cameron A, et al. The rising prevalence of diabetes mellitus and impaired glucose tolerance: the Australian diabetes, obesity and lifestyle study. Diabetes Care 2002; 25: 829-34.
2. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity (Review). Endocr Rev 1998; 19: 491- 503.
3. Evans JL, Goldfi ne ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002; 23: 599-622.
4. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet 1994; 344: 721-4.
5. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996; 19: 257-67.
6. Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 1990; 173: 932-9.
7. Krieger-Brauer H, Kather H. Human fat cells possess a plasma membrane bound H2O2 generating system that is activated by insulin via a mechanism by passing the receptor kinase. J Clin Invest 1992; 89: 1006-13.
8. Habib MP, Dickerson FD, Mooradian. Effect of diabetes, insulin and glucose load on lipid peroxidation in the rat. Metabolism 1994; 43: 1442-5.

9. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.

10. Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 2002; 13: 51-9.

11. Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. Diabetes 2004; 53 Suppl 1: S152-8.

12. Beltowski J. Leptin and atherosclerosis. Atherosclerosis 2006; 189: 47-60.

13. Beltowski J. Role of leptin in blood pressure regulation and arterial hypertension. J Hypertens 2006; 24: 789-801.

14. Halaas J, Gajiwala K, Maffei M, Cohen S, Chait B, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995; 269: 543-6.

15. Dagogo JS, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 1996; 45: 695- 8.

16. Chu NF, Spiegelman D, Rifai N, Hotamisligil GS, Rimm EB . Glycemic status and soluble tumor necrosis factor receptor levels in relation to plasma leption concentrations among normal weight and overweight US men. Int J Obes Relat Metab Disord 2000; 24: 1085-92.

17. Stefanovica A, Kotur-Stevuljeica J, Spasica S, Bogavac-Stanojevica N, Bujisicb N. The influence of obesity on the oxidative stress status and the concentration of leptin in type 2 diabetes mellitus patients. Diabetes Res Clin Pract 2008; 79: 156-63.

18. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzmán M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 2001; 276: 25096-100.

19. Laight DW, Carrier MJ, Anggard EE. Antioxidants, diabetes and endothelial dysfunction. Cardiovasc Res 2000; 47: 457-64.

20. Hu FB, Stampfer MJ. Is type 2 diabetes mellitus a vascular condition Arterioscler Thromb Vasc Biol 2003; 23: 1715-6.

21. Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, et al. Leptin and incident type 2 diabetes: risk or protection?. Diabetologia 2006; 49: 2086-96

22. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984; 310: 341-6.

23. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28: 1039-57.

24. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90: 37-43.

25. McLemore J L, Beeley P, Thorton k, Morrisroe k, Blackwell W, Dasgupta A. Rapid automated determination of lipid hydroperoxide concentrations and total antioxidant status of serum samples from patients infected with HIV. Am J Clin Pathol 1998; 109: 268-73.

26. VanderJagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Vander Jagt DL. Oxidative stress indices in IDDM subjects with and without long-term diabetic complications. Clin Biochem 2001; 34: 265-70.

27. West IC. Radicals and oxidative stress in diabetes. Diabet Med 2000; 17: 171-80.

28. Vessby J, Basu S, Mohsen R, Berne C, Vessby B. Oxidative stress and antioxidant status in type 1 diabetes mellitus. J Intern Med 2002; 251:69-76.

29. Sundarm RK, Bhaskar A, Viljayalingam S, Viswanathan M, Mohan R, Shanmugasundram K R. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci 1996; 90: 255-60.

30. Thérond P, Bonnefont-Rousselot D, Davit-Spraul A, Conti M, Legrand A. Biomarkers of oxidative stress: an analytical approach. Curr Opin Clin Nutr Metab Care 2000; 3: 373-84.

31. Wu J, Lei MX, Chen HL, Sun ZX. Effects of rosiglitazone on serum leptin and insulin resistance in patients with type 2 diabetes. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2004; 29: 623- 6 (Chinese).

32. Segal KR, Landt M, Klein S. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes 1996; 45: 988-91.

33. Muoio DM, Dohn GL, Fiedorek FT, Tapscott EB, Coleman RA. Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 1979; 46: 1360-3.