Original Article
Leptin concentrations in type 2 diabetes and non-diabetes Nigerian-Africans

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Abstract: Most studies on leptin in diabetes mellitus (DM) compared to healthy controls were done in Caucasians, with conflicting findings. Paucity of data on this exists in Nigerian-Africans. Therefore, the study determined plasma leptin concentrations in newly diagnosed type-2 diabetes versus controls and its relation to obesity/demographic-metabolic indices. A cross-sectional comparative study on 154 subjects: 67 diabetes and 87 healthy controls at the Ahmadu Bello University Teaching Hospital, Nigeria. Leptin was determined by the sandwich enzyme-linked immuno-sorbent assay. Mann-Whitney U test, Spearman's Correlation and Step-wise Multiple Logistic Regression analysis of Log-transformed variables determined outcomes. Leptin trended towards lower levels in DM subjects than controls when both sexes were combined, though insignificant ($P=0.12$). Leptin was significantly ($P<0.001$) positively correlated with waist circumference (WC) and body mass index (BMI) in DM (WC, r=0.71; BMI, r=0.84) as well as controls (WC, r=0.46, BMI, r=0.51), respectively. Leptin was significantly ($P<0.001$) higher in females than males, with approximately 2 times Odds of female sex association with log-transformed (Ln_{10}) hyperleptinaemia {Odds Ratio (OR): 1.9, 95% CI, 0.97-3.92, $P<0.001$}. Age was positively (r=0.21, $P=0.05$) correlated to leptin in controls, while fasting blood glucose (FBG) negatively correlated to uncontrolled DM (r=-0.26). Leptin showed no significant ($P>0.05$) correlation to fasting insulin (FI) and HOMA-IR. WC was an independent predictor of Ln_{10}hyperleptinaemia in DM subjects (OR: 1.12, 95% CI, 1.03-1.23, $P=0.01$). BMI showed significant ($P<0.001$) association with Ln_{10}hyperleptinaemia in both subjects. Conclusively, leptin trends towards lower levels but are not different in newly diagnosed DM than controls. The association of leptin with obesity is similar but stronger in diabetes than controls, with no relations to FI and HOMA-IR. WC and BMI are independent predictors of hyperleptinaemia.

Keywords: Leptin, type 2 diabetes mellitus, obesity indices, healthy controls, insulin resistance, Nigerian-Africans

Introduction

There is a global rise in the prevalence of type 2 diabetes mellitus (DM) currently affecting 463 million adults worldwide, with incidence of 1 in 11 adults [1]. Further, the westernization of Nigerian-Africans and the consequent rise of diabetes in Africans {3 in 4 (79%) people live with diabetes in low-middle income countries} [1], poses serious health concerns as diabetes subjects; despite improved anti-diabetes therapy, enhanced glucose monitoring systems and improved patient to physicians access, are vulnerable to short and long term complications of DM inclusive of cardiovascular disease (CVD), renovascular disease, sudden blindness and amputations [2, 3].

Visceral obesity is central to type 2 diabetes mellitus, as the adipocytes release a number of humoral factors called adipokines of which leptin is one amongst others, such as resistin, ghrelin, visfatin, apelin, vaspin, plasminogen activator inhibitor-1, retinol-binding protein-4, tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), low adiponectin levels and omentin, just to mention a few, with consequent insulin resistance [4-6].

Leptin produced from the obesity (ob) gene plays a pivotal role in energy homeostasis, glucose metabolism and body weight regulation [7-11]. Circulating leptin levels show direct relations to the total amount of body fat in the body [12] and correlates positively with waist circum-

ference (WC), waist-hip ratio (WHR) and body mass index (BMI) [3, 8, 9, 12-14]. However, the leptin concentrations in type 2 diabetes and its relation to obesity indices shows conflicting reports globally, hence, still a subject of debate [15]. It targets the medio basal arcuate nucleus of the hypothalamus, inhibiting appetite [4]. Its deficiency or resistance results in hyperphagia, obesity and diabetes mellitus [2, 4, 7]. It is also implicated in the aetiopathogenesis of myriads of other diseases such as metabolic syndrome, inflammatory diseases, atherosclerosis and cardiovascular diseases [2, 16-18]. Hormonal and metabolic factors affect leptin inclusive of insulin, steroid, thyroid and estradiol which stimulate, while testosterone inhibits leptin synthesis [19].

Animal models of leptin deficiency have characteristic progressive insulin resistance and associated neuroendocrine derangements in uncontrolled diabetes mellitus [16]. Further, systemic administration of leptin at normal physiological doses enhanced insulin sensitivity, preventing the development of severe progressive insulin resistance (IR) in rats with uncontrolled diabetes, which was not attributable to leptin-induced changes in food intake or body weight [11, 18, 20].

Considerable interests in leptin's potential treatment of obesity, IR and diabetes have been elicited, with several studies done in Caucasians [11, 21]. There are also conflicting findings on leptin levels in diabetes, with some studies showing lower levels in diabetes subjects than healthy controls [12, 22-25], while some other showed no difference [14, 26-28], yet still others, showed higher levels in diabetes subjects than controls [8, 15, 29, 30]. More so, several factors affect leptin levels inclusive of age, sex, body mass index, central adiposity, insulin levels, insulin sensitivity and anti-diabetes therapy [12, 14].

The indigenous African population have few data on leptin levels in relation to diabetes and controls [28] and it has been suggested that ethnic differences exist on account of possible genetic, environmental and geographical variations [13]. Hence, this study was aimed to determine plasma leptin concentrations in drug-naïve newly diagnosed type 2 diabetes Nigerian-Africans in comparison to healthy controls and further determine its relations to obesity indices and some metabolic parameters.

Material and methods

Design and subjects

A cross-sectional comparative analytical study carried out among 154 subjects inclusive of 67 type-2 diabetes mellitus patients attending the endocrine clinic of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and 87 apparently healthy controls from willing patient escorts, willing staff and hospital employees. The study also adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for an observational study of this nature and complied with the amended Helsinki's declaration.

Inclusion criteria

Newly diagnosed type 2 diabetes mellitus subjects by The World Health Organization (WHO) criteria [31], with history of osmotic symptoms (polydipsia: excessive thirst and intake of >3 litres of water per 24 hours; polyphagia: excessive hunger and increased food intake; polyuria: excessive micturition >3 litres per day) and fasting blood glucose (FBG) $\geq$ 7 mmol/L, who had not been on therapy were included.

Exclusion criteria

Exclusion criteria were diabetes patients on oral anti-diabetes or current insulin or steroid therapy; antihypertensive or lipid lowering drug use [8, 24, 28]; previous history of ketosis; evidence of secondary diabetes viz.: historical evidence of Cushing's or Addison's disease; stroke; pregnancy and use of oral contraceptives; history suggestive of malignancy as well as inflammatory disease states. Clinical and biochemical evidence of liver or kidney disease, personal history of hypertension or cardiac disease, as well as clinical evidence of thyroid disease, were other exclusion criteria. With controls, exclusion criteria were clinical evidence of illness, personal or family history of diabetes mellitus or hypertension, smoking history, current use of medication and engagement in competitive sports.

Study procedure

Information on biodata and anthropometric measures were obtained from all patients and control subjects. Weights in kilogram (Kg) were taken with light clothing to the nearest 0.5 kg.

Heights in metres were taken to the nearest 0.5 cm with subjects standing erect without shoes or head gear. Body mass index (BMI) was derived by dividing the weight by the square of the height (Ht^2) in kilogram per metre square.

Metabolic studies

Following an overnight 10-12 hours fast, commencing between 21.00 to 22.00 hours the preceding night, 5 millilitres of venous blood were drawn from each subject and aliquots placed into fluoride oxalate tubes for blood glucose determination and ethylenediaminetetraacetic acid (EDTA) treated tubes with aprotinin (trasylol®) {500 Kallikrein Inactivator Units per mL (KIU/mL) (USA; Lot No: SLBD9903V; Catalog No: 1001466032)} for insulin assay and other hormonal assay inclusive of plasma leptin. The plasma following centrifugation were stored at -20°C until analysis using the commercially available human insulin enzyme linked immunosorbent assay (ELISA) (DRG instruments Gmbh, Marburg Germany Kat/Cat #: EIA-2935) and leptin ELISA kits (Diagnostic Automation/Cortez Diagnostics Inc., Calabasas, CA 91302, Catalog Number: 1742-6). Samples were analysed on the same day.

The insulin ELISA kit has an inter-assay and intra-assay co-efficient of variation of 5.2% and 4.8% respectively, sensitivity of 99% for human insulin and no cross-reaction with pro-insulin. Serum glucose analysis for fasting and two hours post prandial were done within an hour of collection of sample via the glucose oxidase method. Insulin resistance (IR) values were derived using the homeostasis model assessment (HOMA) method employing the equation below: [31]

IR = (Fasting Plasma Insulin, µIU/ml) × (Fasting Plasma Glucose, mmol/L)/22.5

The figure 22.5 brings the insulin resistance value to 1.0 (insulin sensitivity of 100%) or "normal" subjects. Insulin values were regarded as microunits per millilitre (µIU/mL) while leptin values were regarded as nanogram per millilitre (ng/mL).

Statistical analysis

This was done using the Statistical Package for the Social Sciences (SPSS) version-25 software (IBM). Test for normality of data distribution was via the Kolmogorov Smirnov test. Data was presented as Mean ± Standard Deviation (SD) for numerical variables and Median + Interquartile range for non-parametric data or number (proportion) where appropriate. Independent Student's t test or Mann-Whitney U test determined the difference in two numerical variables for parametric and skewed data respectively. Leptin variables were log-transformed to reduce its skewness. Spearman correlation analysis determined the correlation between log-transformed leptin and demographic-metabolic variables. Further step-wise Logistic Regression analysis was performed to determine further associations. The level of statistical significance in each case was taken as *P≤0.05* at 95% confidence interval.

Ethics approval and consent to participate

Institutional ethical approval was obtained from the Health Research Ethics Committee (ABUTH/HREC/S16), ABUTH, Zaria, Nigeria. Written informed consent from all study participants after full explanation of the purpose and nature of all procedures used were obtained.

Results

Subject participation in the study

There were 75 newly diagnosed type 2 diabetes subjects screened over a 6 months' period. Of these, 8 were excluded on account of 4 having a personal history of hypertension; 2 with previous stroke history and 2 had commenced treatment with insulin. Hence, a total of 67 type 2 diabetes subjects were eligible (**Figure 1**). There were 87 healthy non-diabetes controls also recruited.

Demographic-metabolic characteristics of the study population

The Median age was 50 years. There was no significant (*P>0.05*) difference in age, body mass index (BMI), waist circumference (WC) and fasting insulin (FI) between the diabetes and non-diabetes subjects by Mann-Whitney U test (**Table 1**). There were significantly (*P≤0.001*) higher Median fasting blood glucose (FBG) and homeostasis model assessment - Insulin resistance (HOMA-IR) in the diabetes subjects than non-diabetes controls (**Table 1**). The Median leptin levels showed no significant (*P=0.12*) difference between diabetes subjects and healthy
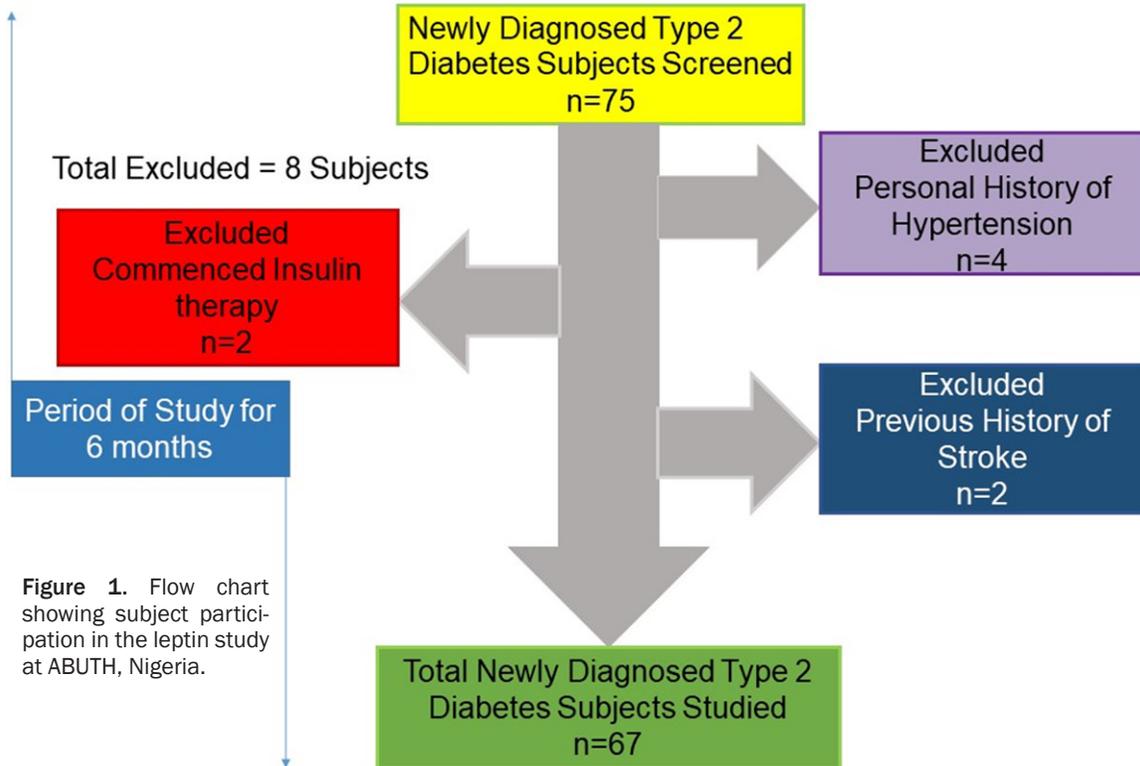
Figure 1. Flow chart showing subject participation in the leptin study at ABUTH, Nigeria.

Table 1. Demographic-metabolic characteristics of the study population

Parameters	Controls (n=87)	Diabetes subjects (n=67)	Total subjects (n=154)	P-Value
Median Age + IQR (Years)	47.5 (36.0, 54.0)	50.0 (45.0, 55.0)	50.0 (38.0, 55.0)	0.10
Median Waist Circumference + IQR (cm)	98 (89.0, 105.3)	100 (89.0, 106.8)	98.5 (88.8, 106.0)	0.33
Median Body Mass Index + IQR (Kg/m²)	29.0 (24.0, 32.0)	28.5 (24.0, 33.0)	29.0 (24.0, 32.3)	0.36
Median Fasting Blood Glucose + IQR	4.9 (4.2, 5.5)	9.9 (6.5, 13.0)	5.6 (4.5, 9.1)	<0.001*
Median Fasting Insulin (µIU/mL)	12.9 (8.9, 20.6)	15.0 (9.0, 21.0)	14.0 (9.0, 21.0)	0.86
Median HOMA-Insulin Resistance + IQR	2.4 (1.7, 4.1)	6.9 (2.8, 10.9)	3.1 (1.9, 6.7)	<0.001*
Median Leptin Levels + IQR (ng/mL)	44.8 (27.9, 62.3)	39.5 (31.6, 61.9)	41.1 (29.6, 63.7)	0.12

Data presented as Median + Interquartile Range (IQR) with 25th and 75th percentiles considered. Mann-Whitney U test determined differences in Parameters between Diabetes Subjects and Healthy Controls. *Level of significance at *P≤0.001*. HOMA: Homeostasis Model Assessment.

controls by Mann-Whitney U test though it trended towards lower levels in diabetes subjects when both sexes were combined (**Table 1**).

Gender differences in demographic-metabolic variables

Both males and female subjects showed no significant (*P>0.05*) differences in age, WC, BMI and FI levels (**Table 2**). However, the Median FBG were significantly (*P<0.001*) higher in both male and female diabetes subjects than their

normal healthy controls. HOMA-IR was also significantly (*P=0.001* & *P<0.001*) higher in male and female diabetes subjects than their normal healthy controls respectively (**Table 2**).

Furthermore, the Median leptin concentrations showed no significant (*P>0.05*) difference between the male and female diabetes subjects than their healthy controls respectively. The female subjects showed significantly (*P<0.001* & *P=0.009*) higher leptin levels than males in both diabetes subjects and normal healthy controls, respectively (**Table 2**).

Table 2. Gender differences in demographic-metabolic parameters between non-diabetes and diabetes subjects

Parameters	Male subjects			Female subjects		
	Non-diabetes	Diabetes	*P*-Value	Non-diabetes	Diabetes	*P*-Value
Median Age (Years)	39 (36, 54)	55 (45, 60)	0.06	49 (38, 55)	50 (45, 53)	0.86
Median Waist Circumference (cm)	95 (89, 102)	95 (85, 107)	0.20	99 (89, 110)	101 (92, 107)	0.86
Median Body Mass Index (Kg/m^2)	28 (24, 31)	27 (22, 32)	0.11	30 (24, 35)	32 (25, 37)	0.44
Median Fasting Blood Glucose (mmol/L)	4.7 (4.0, 5.4)	10 (6.6, 13.0)	<0.001**	4.9 (4.3, 5.6)	8.7 (5.5, 13.5)	<0.001**
Median Fasting Insulin (µIU/L)	15.0 (9.9, 21.0)	15.0 (9.0, 21.0)	0.49	11.5 (6.3, 20.5)	15.0 (7.7, 21.5)	0.53
Median HOMA-Insulin Resistance	2.5 (1.9, 4.7)	6.2 (2.6, 10.2)	0.001**	2.4 (1.6, 4.1)	7.3 (2.7, 11.9)	<0.001**
Median Leptin (ng/mL)	‡33.8 (24.0, 65.0)	†33.6 (21.5, 41.2)	0.30	‡51.9 (35.3, 79.6)	†61.6 (34.5, 85.0)	00.68; ‡0.009* ††<0.001**

Data presented as Median + Interquartile Range with 25th and 75th percentiles considered. Mann-Whitney U tests for difference in Numerical. Variables between Diabetes and Non-Diabetes subjects. WC: Waist Circumference; BMI: Body Mass Index; FBG: Fasting Blood Glucose; FI: Fasting Insulin; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance. *Level of significance at *P*≤0.01. **Significant at *P*≤0.001. ‡†Each Pair with a common superscript symbol is significant at a particular *P*-value by Pairwise Comparison.

Table 3. Correlation between plasma leptin levels and demographic-metabolic parameters among diabetes subjects and normal controls

Parameters	Non-Diabetes (n=87)		Diabetes Subjects (n=67)		Total Sample (n=154)	
	r	P-Value	r	P-Value	r	P-Value
Age (Years)	0.21	0.05*	0.001	0.99	0.09	0.25
Sex	0.28	0.008**	0.49	<0.001***	0.39	<0.001***
Waist Circumference (cm)	0.46	<0.001***	0.71	<0.001***	0.56	<0.001***
Body Mass Index (Kg/m²)	0.51	<0.001***	0.84	<0.001***	0.66	<0.001***
Fasting Blood Glucose (mmol/L)	-0.01	0.92	-0.26	0.03*	-0.17	0.03*
Fasting Insulin (µIU/L)	0.15	0.19	0.06	0.65	0.07	0.44
HOMA-Insulin Resistance	0.16	0.15	-0.17	0.20	-0.03	0.73

Spearman Correlation Analysis. *Level of significance at $P \leq 0.05$. **Significant at $P \leq 0.01$. ***Significant at $P \leq 0.001$.

Correlation of plasma leptin with demographic-metabolic variables

Spearman's Correlation analysis showed significant ($P<0.001$) positive correlation of plasma leptin with obesity indices both central and generalized obesity, with stronger correlation coefficient among the diabetes subjects than non-diabetes controls (**Table 3**). Likewise, plasma leptin was significantly ($P<0.001$) positively correlated with sex in diabetes subjects and healthy controls as well as all subjects combined. FBG was however, significantly ($P=0.03$) negatively correlated to plasma leptin in diabetes subjects and the whole sample population (**Table 3**). There was no significant ($P>0.05$) correlation of leptin with fasting Insulin and HOMA-IR (**Table 3**).

Association of log-transformed plasma leptin with demographic-metabolic variables

Binary Logistic Regression analysis showed that log-transformed hyperleptinaemia (Ln_{10}hyperleptinaemia) was significantly ($P<0.001$) associated with WC in healthy controls, diabetes subjects as well as all subjects combined, with higher Odds in diabetes subjects (**Table 4**). There was also significant ($P<0.05$) association of leptin with BMI, with a 7 times Odd amongst diabetes subjects. Generalized obesity by BMI ≥30 kg/m² was significantly ($P<0.001$) associated with hyperleptinaemia in the healthy controls and all subjects combined with high Odds (**Table 4**). Fasting blood glucose showed significant ($P=0.047$; $P=0.008$, respectively) association with hyperleptinaemia in the diabetes subjects and all subjects combined. Sex was also associated ($P=0.03$) with hyperleptinaemia in

all subjects, with higher Odds in the females than males. There was no significant ($P>0.05$) association of Ln_{10}hyperleptinaemia with FI and HOMA-IR (**Table 4**).

Further step-wise Multiple Logistic Regression analysis showed that WC was an independent predictor of hyperleptinaemia in non-diabetes, diabetes and all subjects combined with higher Odds among diabetes subjects following age, sex, FBG, FI and HOMA-IR adjustments (**Table 4**). BMI showed similar trends; however, the association following adjustments was for all subjects combined, with generalized obesity ≥ 30 kg/m² being significantly ($P<0.001$) independently associated with hyperleptinaemia with 7 times Odd (**Table 4**).

Discussion

Findings from this study has shown that there is no difference in leptin concentrations in newly diagnosed type-2 diabetes subjects than healthy controls who did not differ from each other age-wise and by obesity indices, even though leptin trended towards lower levels in the diabetes subjects when both sexes were combined. This is consistent with previous reports which showed no difference [9, 14, 26-28], corroborating previous findings even as far back as the nineties [27]. Some other reports showed significantly ($P<0.05$) lower leptin levels in diabetes than controls, somewhat similar to the trend observed in this study even though insignificant ($P>0.05$) [13, 22-24].

Insulin is required for the synthesis of triacylglycerol and its storage in adipose tissues. Therefore, in the absence of insulin therapy in

Table 4. Comparative relationship between log-transformed plasma leptin and demographic-metabolic parameters in diabetes subjects and controls

Parameters	Non-diabetes (n=87)			Diabetes (n=67)			Total (n=154)		
	Odds ratio	95% CI	*P*-Value	Odds ratio	95% CI	*P*-Value	Odds ratio	95% CI	*P*-Value
Unadjusted Model									
Age (Years)	0.99	0.93-1.05	0.73	1.03	0.98-1.09	0.26	0.98	0.94-1.02	0.41
Sex	2.70	0.64-11.71	0.18	2.87	0.71-11.6	0.14	3.01	1.11-8.17	0.03*
Male	0.64	0.37-1.08	0.16	1.43	0.97-2.11	0.13	0.65	0.47-0.88	0.025*
Female	1.70	0.67-4.50		0.50	0.18-1.40		1.90	0.97-3.92	
Waist Circumference (cm)	0.91	0.86-0.98	0.009**	1.10	1.04-1.20	0.002**	0.91	0.87-0.94	<0.001***
Body Mass Index (Kg/m²)	0.80	0.67-0.95	0.01*	7.40	1.43-37.9	0.017*	0.81	0.75-0.88	<0.001***
General Obesity, BMI ≥ 30	5.10	0.80-33.8	0.012*	------	------	0.99	3.98	2.06-7.71	<0.001***
General Obesity, BMI <30	0.53	0.39-0.73					0.48	0.37-0.62	
Fasting Blood Glucose (mmol/L)	1.20	0.55-2.50	0.70	0.87	0.76-1.00	0.047*	1.14	1.04-1.30	0.008**
Fasting Insulin (µIU/L)	1.00	0.95-1.07	0.86	1.00	0.92-1.08	0.91	1.00	0.96-1.05	0.86
HOMA-Insulin Resistance	0.95	0.69-1.32	0.76	0.92	0.80-1.06	0.23	1.08	0.97-1.20	0.17
Adjusted Model									
†Waist Circumference (cm)	0.91	0.84-0.98	0.009**	1.10	1.03-1.20	0.003**	0.91	0.88-0.95	<0.001***
‡Waist Circumference (cm)	0.90	0.82-1.00	0.04*	1.12	1.03-1.23	0.01*	0.92	0.89-0.96	<0.001***
†Body Mass Index (Kg/m²)	0.11	0.01-0.93	0.01*	0.88	0.76-1.01	0.07	0.82	0.75-0.89	<0.001***
‡Body Mass Index (Kg/m²)	0.15	0.02-1.50	0.11	0.75	0.82-1.06	0.10	0.83	0.76-0.91	<0.001***
†General Obesity, BMI ≥ 30	------	------	0.99	------	------	0.99	7.50	3.20-17.8	<0.001***
‡General Obesity, BMI ≥ 30	------	------	0.99	------	------	0.99	7.13	2.80-18.2	<0.001***

Step-Wise Multiple Binary Logistic Regression Analysis. BMI: Body Mass Index; WC: Waist circumference; HOMA: Homeostasis Model Assessment. †Adjusted for Age and Sex; ‡Adjusted for Age, Sex, FBG, FI and HOMA-IR. *Level of significance at *P*≤0.05. **Significant at *P*≤0.01. ***Significant at *P*≤0.001.

uncontrolled DM, body fat stores are depleted and the rapid progressive loss of body fat stores results in amelioration of plasma leptin concentrations [11, 33]. Hence, uncontrolled DM is characterised by hyperglycaemia and excessive food intake, with insulin and leptin deficiency being implicated [11]. This study showed that there was uncontrolled diabetes as FBG was significantly ($P<0.001$) higher in DM subjects than healthy controls, but the Median BMI of the DM subjects was not lower than that of the healthy controls, neither was there any difference in insulin levels. This may account for the lack of significant reduction in leptin levels in DM subjects in this study. More so, the FBG showed weak negative correlations to hyperleptinaemia in diabetes subjects meaning that leptin trended towards higher levels in diabetes subjects with low glucose levels, supporting the evidence that suggests that leptin has glucose lowering effects in uncontrolled DM, another model of acquired leptin deficiency [33]. Evidence suggests that the glucose lowering effects of leptin in uncontrolled diabetes are accompanied by normalization of plasma hyperglucagonaemia, which is one of the hallmarks of DM [34].

Further, the altered fat distribution in diabetes [9], with subjects having more of visceral obesity and less of subcutaneous fat, may be a possible explanation of the lower leptin levels in diabetes as adduced by some study [24], as visceral fat produces less leptin than subcutaneous fat. This was supported by the higher Odds of generalized obesity being associated with hyperleptinaemia even following step wise Logistic Regression analysis. Relative defective pancreatic ß-cell function and associated insulin deficiency in type 2 diabetes, with consequently higher HOMA-IR in diabetes than healthy controls [22, 24], might further explain the trend towards lower leptin levels in DM subjects than healthy controls, as insulin is an important stimulator of leptin synthesis [22, 24].

Contrary reports exist, in which higher leptin levels were found in diabetes subjects than healthy controls [29, 30]. The reason for the conflicting reports globally may be attributed to the patient selection criteria, chronically elevated plasma insulin levels & insulin resistance, anti-diabetic therapy and diabetic nephropathy

[29]. Other factors may be, differences in age, extent of obesity, gender as well as ethnicity [24]. Most of the studies with hyperleptinaemia in diabetes compared to healthy controls recruited patients who had been on treatment [8, 24, 29] unlike the newly diagnosed in this study. Anti-diabetes therapy such as glibenclamide, sulphonylurea or insulin therapy, increase leptin blood levels [35]. Also, insulin resistance is the underlying mechanism of type 2 DM and leptin secretion is induced in a dose-dependent fashion [11, 29]. Hence, with IR and chronic hyperinsulinaemia, type 2 DM patients are expected to develop a hyperleptinaemic state [29]. However, acute hyperleptinaemia may not cause insulin resistance [29]. More so, hyperleptinaemia can be a consequence of impaired renal function in type 2 diabetes subjects [36]; however, its mechanisms: whether due to reduced renal clearance, increased production or increased leptin resistance is yet to be clarified [29]. Subjects with renal impairment were excluded *ab initio* from this study hence may be another reason for the lack of higher leptin levels in DM than healthy controls.

Furthermore, was the positive correlation of leptin with obesity indices (both BMI and WC) which was similar, albeit stronger in diabetes subjects than healthy controls. This was further buttressed by the high Odds of association of obesity indices with log-transformed hyperleptinaemia especially among diabetes subjects. Central obesity by WC proved to be an independent predictor of hyperleptinaemia following step-wise adjustments for age, sex, FBG, FI and HOMA-IR, with higher Odds in diabetes subjects than healthy controls. BMI only proved to be independently associated with hyperleptinaemia in all subjects combined, with high Odds. Previous studies have documented strong positive correlation of leptin with body mass index, body fat percentage by bioimpedance, waist circumference and waist-hip ratio [3, 8, 9, 12-14, 27, 28]. This supports the existing evidence that the fat stores/adipose cells release adipocytokines especially leptin which is anti-insulinic and can result to insulin resistance seen in type 2 diabetes [28] as observed in this study. The moderate correlation of leptin with obesity indices in healthy controls might be due to the influence of obesity, as leptin levels are higher in obese than non-obese sub-

jects [4]. However, further Regression analysis showed that the Odds of such an association among healthy controls were lower than 1, signifying a protective rather than an adverse effect of hyperleptinaemia in healthy subjects and vice versa for the diabetes subjects.

Some contrary report documented a lack of correlation of leptin with obesity indices in diabetes except for their healthy controls and the reason adduced was the possibility of loss of normal mechanism of leptin regulation with advancing disease, hence newly diagnosed subjects were recommended to be utilized in subsequent studies [25].

Sex was also shown to be associated with hyperleptinaemia in this study with higher Odds in females. Consistent with previous reports, females showed one and a half to two times higher leptin levels than males. This may be attributable to the oestrogenic stimulation of leptin production; precisely 17 ß-oestradiol stimulation of leptin messenger ribonucleic acid (mRNA) [22, 24] and androgenic inhibition of same, as leptin has been documented to negatively correlate with testosterone levels [22]. Other reasons for this sex difference may be the disparity in body fat distribution, with females possessing more of subcutaneous fat than males who tend to have more of visceral adiposity [24]. It's been reported that subcutaneous fat produces more leptin than visceral adiposity [24].

On a further note, age was also shown to be weakly positively correlated with leptin levels among the healthy controls, suggesting higher levels with older age than younger age which is consistent with findings from some previous studies [13, 27]. This however, did not persist following further Logistic Regression analysis. Some other study reported contrary findings showing declining leptin levels with older age and higher levels in younger age, attributed to loss of subcutaneous tissue with aging [19].

Similar to previous reports [8, 14], leptin showed no significant relationship to FI and HOMA-IR. Some other studies found positive relationship with HOMA-IR [2], [29] attributable to increased fat mass, though in the latter study, IR was not a predictive factor of hyperleptinaemia [2]. The disparity in the present study may be the selection criteria of newly

diagnosed, as acute hyperleptinaemia may not translate to insulin resistance [29] or perhaps ethnic differences or possibly the interaction of other inflammatory molecules released alongside leptin which were not assayed here hence, cannot objectively suffice.

Conclusively, strategies that enhance blood brain barrier permeability of leptin are being developed and advocated in Industrialized Nations for the treatment of leptin resistance, obesity, diabetes and metabolic syndrome [11, 20, 21, 37]. These include structural modification of leptin, leptin mimetic and analogues, leptin receptor agonists, as well as development of combination therapy with molecules that ameliorate leptin resistance and its sensitivity [37]. Future directions for Africans especially sub-Saharan Africans should encompass further clinical studies in this direction.

Conclusion

Leptin concentrations trend towards lower levels but are not different in newly diagnosed DM than healthy controls. The association of leptin with obesity is similar but stronger in diabetes than non-diabetes subjects. Leptin concentrations are higher in females than males irrespective of diabetes status. It also shows positive relations to obesity indices and female sex in both groups, age in healthy controls, as well as negative correlation to FBG but no relations to FI and HOMA-IR. WC and BMI are independent predictors of hyperleptinaemia.

Limitations

The cross-sectional nature of this study assayed single leptin measurements and did not allow for serial or multiple measurements as leptin is unstable and known to have a circadian pattern [29]. However, the use of aprotinin in its storage was an added advantage, as this inhibits the proteinases enzymes that biodegrade leptin molecules, thereby enhancing its stability. Larger population based studies across the geopolitical zones of Nigeria as well as Africa, incorporating larger sample sizes are advocated, however, previous studies with valid conclusions used similar or smaller sample sizes. The use of the hyperinsulinaemic euglycaemic clamp and steady state plasma glucose estimation which are rather cumbersome, as gold standard for estimation of insulin resis-

tance was not done. However, the HOMA-IR used in this study is an easy, widely utilized and validated tool and may be useful for large epidemiologic studies.

Recommendations

Routine check of obesity indices via WC and BMI as well as leptin assays should be encouraged in African blacks. Exercise schemes, lifestyle modification and routine health education should be advocated and implemented by the government and policy makers in the health-care systems, as a means to prevent obesity, type 2 diabetes and its consequent cardiovascular complications and mortality. Molecular mechanisms of leptin polymorphisms should be assessed in Nigerian-Africans in further longitudinal studies. Randomised controlled studies on the effect of leptin adjunct therapy in treatment of newly diagnosed type 2 diabetes Africans are also advocated.

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Disclosure of conflict of interest

None.

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