Resistin Levels In Healthy Non-Diabetes Nigerian-Africans: Effect Of Obesity The ABU Adiporesistin Survey

Obiageli UZOAMAKA Onyemelukwe (obiageliuo629@gmail.com)
Ahmadu Bello University Teaching Hospital https://orcid.org/0000-0002-3274-3154
Dimie Ogoina
Niger Delta University
Geoffery Chukwubuike Onyemelukwe (MON)
Ahmadu Bello University Teaching Hospital

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Abstract

Background Conflicting findings exist on the mechanisms of resistin in obesity and insulin resistance among white populations, as data on these in black Africans are scarce, on account of genetic/geographical differences. Hence, the study aimed to determine plasma resistin levels in Nigerian-Africans and explore its relationship with obesity and selected cardiometabolic risks.

Materials and Methods A cross-sectional study on 87 randomly-selected non-diabetes Nigerians of both genders allocated into three study groups: 24 normal weight; 23 overweight and 40 obese groups by the WHO criteria. Fasting Insulin (FI), Homeostasis Model Assessment-Insulin resistance (HOMA-IR) and obesity indices were determined. Plasma resistin was measured via enzyme linked immunosorbent assay. One-way Kruskal-Wallis test determined cardiometabolic parameters across the groups with Spearman's correlation assessing relationships.

Results Resistin levels were higher in overweight and obese than normal weight subjects {6.6±3.8, 6.7±4.2 versus 5.6±2.9 μIU/mL, ($p<0.001$)}, with highest concentrations in severely obese than mildly and non-obese subjects {(7.4±5.6 versus 6.7±4.2 versus 6.05±3.0 μIU/mL ($p<0.001$, One-way Kruskal-Wallis). However, resistin showed no significant ($p>0.05$) correlations to HOMA-IR, FI and obesity indices. The higher HOMA-IR found in overweight and obese than normal weight subjects (3.7±3.5, 2.9±2.4 vs. 2.1±0.4, $p<0.001$), was positively correlated to obesity and FI, which also increased from mild to severe BMI categories.

Conclusion Higher resistin concentrations are found in severe obesity among non-diabetes black Africans, but its lack of correlation with insulin resistance and obesity indices may suggests possible interplay of other pro-inflammatory cytokines or hormones which may be evaluated in further studies.

Introduction

Resistin otherwise known as Found in the Inflammatory Zone three (FIZZ3) or adipose tissue-specific secretory factor (ADSF) belongs to a family of cysteine-rich C-terminal protein called resistin-like molecules (RELMS) which has its RETN gene encoded in chromosome 19 in humans.[1] First discovered in the white adipose tissue of mice, its expression as a 114-amino-acid peptide produced predominantly in murine adipocytes[2] is structurally and functionally different from its human expression as a 108 amino-acid polypeptide in adipocytes, placenta,[3] gut,[4] pancreatic cells,[1,5] as well as its abundant expression in mononuclear cells & macrophages.[5] Higher levels of expression of both gene and protein in central adipose tissue depots, supported a note for resistin in linking central obesity to diabetes with recent meta-analysis showing the positive correlation of resistin with insulin resistance in type 2 DM and obese subjects with hyperresistinaemia but not in those with normal circulating resistin levels.[1,5–8] On the contrary, conflicting data exist regarding resistin and its linkage to obesity and insulin sensitivity and or resistance, as some studies have reported low resistin messenger ribonucleic acid (mRNA) expression in isolated human adipocytes and associated lack of correlation with obesity or insulin resistance, thereby making its role in insulin resistance rather unclear.[1] Furthermore, some other study found no difference in serum resistin levels between lean healthy and obese insulin resistant non-diabetes as well as type 2 diabetes adolescence.[9] More so, although resistin levels increased in the second trimester of pregnancy, there were paradoxically lower values found in gestational diabetes mellitus.[3]

Resistin is also expressed in cardiomyocytes; its over-expression alters cardiac contractility and via activation of the insulin receptor substrate–1/mitogen-activated protein kinases (IRS–1/MAPK) pathway, promotes cardiac hypertrophy.[10,11] Endothelial cell function and homeostasis may also be directly affected by adipocyte-derived hormones: Reilly and coworkers showed that in both non-diabetes and diabetes subjects, plasma resistin levels were not only associated with metabolic and inflammatory markers viz.: {tumour necrosis factor alpha (TNF-alpha); interleukin (IL–6), lipoprotein associated phospholipase A} but correlated with coronary artery calcification which is a quantitative measure of atherosclerosis.[11,12] Resistin stimulates endothelin–1 (ET–1), vascular cell adhesion molecule–1 (VCAM–1) and macrophage chemoattractant protein–1 (MCP–1), contributing to endothelial cell dysregulation and consequent cardiovascular disease.[13] It has also been shown to upregulate the expression of adhesion molecules inclusive of VCAM–1, CAM–1 and long pentraxin 3 (PTX 3) in human aortic endothelial cells.[14]

Obesity is a strong risk factor for later onset of type 2 diabetes and may often occur concurrently.[5] It has been documented that 60–90% of all type 2 DM patients are or have been obese, with the World Health Organization (WHO) coining the duet, a 21st century pandemic.[5] A parallel rise in the prevalence of obesity and diabetes has attracted the term "diabesity" and studies have reported underlying mechanisms in which central obesity leads to insulin resistance and consequent diabetes.[1,15] The fat cells release pro-inflammatory adipocytokines such as leptin, visfatin, apelin, ghrelin, angiotensinogen, adiponectin, TNF-alpha, plasminogen activator inhibitor–1 and resistin which underlies the inflammation and IR linking obesity to metabolic syndrome.[11–12,16–17] Significant correlations between resistin and IL–6, intercellular adhesion molecule one (ICAM–1) have been documented in obese subjects while lipopolysaccharide (LPS), IL–1, TNF-alpha and IL–6 strongly stimulate resistin expression in human mononuclear cells.[1,11–12,16–17]

Furthermore, while several studies regarding biomarkers and mechanisms underlying obesity and IR have been elucidated in western populations; [18] studies regarding this in black African populations especially Nigerians are lacking, bearing in mind the current rise in obesity, type - 2 diabetes and cardiovascular diseases.[19] Hence, there is a need for more data from different ethnic groups, bearing in mind the impact of genetic and geogaphical differences on resistin expression which can be subject to ethnic variations.[1,16,20] Hence, the study aimed to determine baseline plasma resistin levels in apparently healthy non-diabetes Nigerian-Africans and explore its relationship with obesity and selected cardiometabolic risk factors.

Methods

Study Design & Setting

The study was a cross-sectional study carried out at the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria having obtained Institutional ethical approval from the Health Research Ethics Committee (HREC), ABUTH, Zaria, Nigeria. Written informed consent from all study participants after full explanation of the purpose and nature of all procedures used were obtained. The study complied with the amended Helsinki's declaration and involved a total of 87 apparently healthy non-diabetes subjects randomly selected from willing patient escorts, hospital employees and willing staff of same hospital. The study also adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for an observational study of this nature.

Sample size

This was determined by the Fisher's statistical formula for sample size for descriptive studies viz-a-viz: $N = Z^2 pq/d^2$: where N = Sample size; Z = Standard deviation score at 95% = 1.96; p = Prevalence of obesity in Nigeria from meta-analysis with range from 8.1% to 22.2% [21] as studies on resistin levels in black African population are lacking. Therefore, p = 0.08; while q = Complimentary probability (1 - p) = 1 - 0.08 = 0.92; d: Error margin = 5%. Substituting, $N = (1.96)^2 \times 0.08 \times 0.92 / (0.05)^2 = 3.8 \times 0.08 \times 0.9 / 0.003 = 90$. Hence, a sample size of 90 was calculated and rounded up to 100 to take care of 5—10 % attrition rate. However, 13 subjects were excluded hence a sample of 87 healthy non-diabetes subjects were analysed.

Inclusion Criteria

Adults greater than 20 years with no family history of hypertension or diabetes; no clinical history of cardiorespiratory, renal or liver disease as well as no neurological disease (stroke/heart attack) in the previous 36 months.[16] Subjects with stable weight over 3 months prior to the study and no history of diabetes with FBG < 7.0 mmol/L, were also included. Fasted state (last meal was 10–12 hours) prior to blood sampling. All subjects with human immunodeficiency virus (HIV), Hepatitis Bsurface antigen (HBsAg) and Hepatitis C viral antibody (HCV Ab) negativity and normal serum electrolyte, urea and creatinine (Creatinine <1.5 mg/dL) were also included.

Exclusion Criteria

Subjects were excluded on account of the following: Clinical and or laboratory evidence of any illness; chronic pro-inflammatory diseases like arthritis or rhinitis;[17] smoking >20 cigarettes per day in the past one year/ one pack year smoking; clinical evidence of endocrine diseases (hypo or hyperthyroidism);[9] concurrent use of any form of medications such as psychoactive drugs, steroids, metformin or insulin injectable for obesity therapy in the preceding 7 days;[22] engagement in competitive sports or activities and such factors which interfere with insulin secretion and action as well as pregnant or breastfeeding women.[9]

Study Procedure

Sample Collection and Anthropometric Measurements

Socio-demographic information was collected by interviewer administered questionnaire by four trained medical doctors. Body weight in kilogram (Kg) was measured with light clothing and no shoes, to the nearest 0.5 kg. Height in meters (m) was taken with a stadiometer to the nearest 0.5 cm with subjects standing erect without shoes or head gear.[23] Body mass index (BMI) was derived by dividing the weight by the height (Ht^2) in accordance with the WHO recommendation.[23] Waist circumference (WC) was measured to the nearest 1 cm, at a level parallel to the floor, midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid-axillary line while the subjects expired gently.[24] This was done with a non-stretch one cm wide measuring tape wrapped snugly around the subjects without any constricting effect and with the tape level and parallel to the floor at the measurement point. The subjects were standing upright with their arms relaxed at their side, feet spread apart and body weight evenly distributed during the procedure.[24] BMI was classified based on the WHO criteria viz.:[25] Normal (BMI 18.5–24.9 kg/m^2); overweight (BMI 25.0–29.9 kg/m^2) and obese (BMI ≥ 30.0 kg/m2). General obesity was further graded into mild obesity (BMI 30.0–34.9 kg/m^2); moderate (BMI 35.0–39.9 kg/m^2) and severe obesity (BMI ≥ 40.0 kg/m^2).[25] Blood pressure was measured twice after a 10 minutes rest in the recumbent position, standing and sitting with a random zero mercury sphygmomanometer and averaged.

Biochemical and Hormonal Assays

Following an overnight fast (10–12 hours) commencing from 21.00 to 22.00 hour of the previous night, 5mls of venous blood was drawn from each subject into ethylenediaminetetraacetic acid (EDTA) treated bottles, in which 200 KIU (Kallikrein Inactivator Units) of Aprotinin (trasylol®) {USA; Lot No: SLBD9903V; Catalog No: 1001466032} had been previously added. This was centrifuged at 1800 revolution per 20 minutes and plasma separated within 1–2 hours and divided into aliquots in cryovials for plasma insulin and resistin assay. This was stored at - 20^oC until final analysis at the Immunology laboratory of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Plasma glucose was analysed within an hour of collection via the glucose oxidase method while the serum electrolyte, urea and creatinine (SE/U/Cr) were determined via the Chemray 120 automated clinical chemistry auto-analyser at the Chemical Pathology Laboratory of ABUTH, Zaria, Nigeria.

Plasma Resistin Assay

Resistin assays were performed using commercially available enzyme linked immunosorbent assay, ELISA, (Assay Pro) assay Max Human: Resistin ELISA kit (ER 1001, Lot Number: 0257822). The procedure was done according to the manufacturer's manual. Intra-assay and inter-assay coefficients of variation were 4.07% and 7.2% respectively. The reference range for plasma resistin was 4 - 12 ng/mL.[16]

Plasma Insulin Assay

Plasma insulin assays were performed using commercially available ELISA human insulin kit (DRG instruments GmbH, Marburg, Germany with Catalog Number: EIA–2935). The assay kit has an inter-assay and intra-assay coefficient of variation of 5.2% and 4.8% respectively; sensitivity of 90% for human insulin and no cross-reaction with pro-insulin. Insulin resistance values were derived from the formula devised by Mathew and coworkers using the Homeostasis Model Assessment (HOMA) and reproduced by Bonora *et. al.* viz-a-viz:[26]

HOMA-IR = *Fasting plasma Insulin, FPI (μU/mL) x Fasting plasma glucose, FPG (mmol/L)*

22.5

Insulin Resistance was classed by a HOMA-IR ≥ 2.0 for African migrants. [27]

Statistical Analysis

Data was validated and analyzed via Statistical and Products Service Solutions (SPSS) version–22 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were summarized as Mean and Standard Deviation (Mean ± SD) and range (Minimum - Maximum). The Kolmogorov-Smirnov test was used to determine the normality of data distribution. Independent Student's t test determined the sex differences in numerical variables. Three or more Independent numerical variables with normality and equal variances were analysed via One-way Analysis of Variance (ANOVA) with Post-hoc Bonferroni test. When data was not normally distributed with equal variances observed, the Kruskal-Wallis test with Pairwise comparison was used. Two Independent Non-parametric numerical variables were analysed via the Man Whitney U test. Spearman Correlation analysis was used for correlations relationships between plasma resistin and other cardio-metabolic parameters. Differences between groups were considered significant at $p \leq 0.05$ at 95% confidence interval.

Results

Subject Participation

A total of 100 healthy volunteers were screened by simple random sampling at the MOPD of ABUTH, Zaria, of which 13 were excluded on account of the following: 2 were incidentally found to be HIV positive, 3 HBsAg positive, 4 with FBG ≥ 7.0 mmol/L, 4 with family history of diabetes and hypertension, 1 with I year history of previous stroke without neurological deficit, 1 with background history of arthritis {Figure 1}. Hence, 87 non-diabetes adults (39 males and 48 females) aged 26–75 years (Mean age, 46 ± 10.7 years) were found eligible {Figure 1}. These were randomly assigned into 3 groups consisting of 24 normal weight (BMI, 18.5–24.9 kg/m^2) (11 males & 13 females); 23 overweight (BMI, 25–29.9 kg/m^2) (12 males, 11 females); and 40 obese subjects (BMI, ≥ 30kg/m^2) (16 males, 24 females) {Figure 1}.

Characteristics of the Study Population by Gender

The Mean age of the study population was 46.0 ± 10.7 years with no significant (*p > 0.05)* gender differences. Likewise, the mean waist circumference (WC), body mass index (BMI) and fasting blood glucose showed no significant (*p > 0.05)* difference between the males and females (Table 1). The fasting insulin and plasma resistin levels appeared higher in males than females but this was statistically (*p > 0.05)* insignificant. Homeostasis model assessment insulin resistance (HOMA-IR) also showed no significant (*p > 0.05)* difference between both sexes (Table 1).

Table 1
Characteristics of the Study Population by Gender

PARAMETERS	Male n = 39	Female n = 48	Total n = 87	P-VALUE
Age (Years)	45 ± 10.7	47.0 ± 10.9	46.0 ± 10.7	NS
Waist Circumference (cm)	98.0 ± 6.9	99.0 ± 9.2	98.0 ± 8.1	NS
Body Mass Index (kg/m^2)	29.4 ± 2.4	30.4 ± 1.6	29.9 ± 2.0	NS
Fasting Blood Glucose (mmol/L)	4.9 ± 1.2	4.9 ± 0.8	4.9 ± 0.9	NS
Fasting Insulin (µU/mL)	14.0 ± 9.7	15.0 ± 9.8	15.0 ± 9.6	NS
Insulin Resistance	2.3 ± 1.4	2.4 ± 1.2	2.3 ± 1.3	NS
Plasma Resistin (ng/mL)	6.42 ± 3.8	6.31 ± 3.9	6.36 ± 3.8	NS

Difference between Males and Females by Independent Student's t-test. NS: Non - significant at $p > 0.05$

Demographic, Anthropometric & Laboratory Characteristics of the Study Population

The demographic, anthropometric and laboratory characteristic of the study population with respect to WHO classification of BMI and gender are as shown in Table 2. The mean ages and fasting blood glucose showed no statistically significant (*p>0.05)* difference between the total normal, overweight and obese subjects by One-way Analysis of Variance with Post hoc Bonferroni test as well as across the genders in all three categories. However, the mean WC and BMI showed significant (*p<0.001,* One-way ANOVA) difference among the three BMI categories with highest levels in the obese subjects, which was significantly (*p<0.001* respectively, Post hoc Bonferroni) greater than that of overweight as well as normal healthy Non-diabetes subjects (Table 2).

Furthermore, there was a significant (*p < 0.001)* difference in fasting insulin and insulin resistance across the three BMI categories by One-way Kruskall Wallis test; the actual difference by Pairwise comparison was the significantly (*p<0.001, p = 0.003)* higher fasting insulin and HOMA-IR levels, respectively in overweight than normal weight subjects as well as obese (fasting insulin, *p<0.01;* HOMA-IR, *p = 0.02)* than normal weight subjects (Table 2). There was no significant (*p>0.05)* difference in fasting insulin and HOMA-IR levels between the overweight and obese subjects. Likewise, plasma resistin levels showed significant difference across the three BMI categories by One-way Kruskall Wallis test, with highest levels found in the obese subjects, which was significantly higher than normal weight subjects but showed no difference with overweight subjects (Table 2)

Table 2

Demographic, Anthropometric & Laboratory Characteristics of the Study Population by Gender and Body Mass Index

PARAMETERS	NORMAL WEIGHT n=24 (27.6%)			OVERWEIGHT n=23 (26.4%)			OBESE n=40 (46.0%)			P-VALUE
	Male n = 11 (45.8%)	Female n = 13 (54.2%)	Total n = 24 (100%)	Male n = 12 (52.2%)	Female n = 11 (47.8%)	Total n = 23 (100%)	Male n = 16 (40.0%)	Female n = 24 (60.0%)	Total n = 40 (100%)	
AGE (Years) (Min - Max)	44 ± 13.5 30-70	48 ± 12.3 28-70	**47 ± 12.7 28-70**	46 ± 9.1 35-58	46 ± 11.6 26-56	**46 ± 10.1 26-58**	45 ± 10.3 28-60	46 ± 10.2 30-75	**45 ± 10.1 28-75**	NS
WC (cm) (Min - Max)	86.5±5.6 72-92	89.2±1.0 69-100	**87.9±1.0^{a} 69-100**	99.7±10.1 80-120	95.6±9.2 81-110	**97.8±9.7^{b} 80-120**	107±17.3 89-160	108±10.4 84-130	**108±13.4^{c} 84-160**	<0.001
BMI (kg/m^2) (Min - Max)	23.2±2.0 19.9-24.8	23.6±1.7 18.9-24.9	**23.4±1.8^{a} 18.9-24.9**	28.8±0.7 27.7-29.9	28.9±1.2 26.7-29.9	**28.8±1.0^{b} 26.9-29.9**	34.1±5.8 30.4-52.4	35.9±6.1 30.2-55.8	**35.2±5.9^{c} 30.1-55.8**	<0.001
FBG (mmol/L) (Min - Max)	4.2 ± 0.9 3.2-6.2	5.1 ± 0.8 3.6-6.8	**4.8 ± 0.7 3.2-6.2**	5.2 ± 1.2 3.0-6.9	4.8 ± 0.9 3.0-6.6	**4.9 ± 1.0 3.0-6.9**	4.7 ± 0.7 3.0-5.9	4.9 ± 0.8 3.0-6.2	**4.8 ± 0.7 3.0-6.2**	NS
†Fasting Insulin (Min - Max)	11.9±5.2 3-19.5	10.7±2.6 6.3-16.4	**10.7±2.6^{a} 3-19.5**	21.4 ±16.6 1.5-63	11.1±10.4 1.5-29.2	**16.5±14.6^{ab} 1.5-63**	12.4±9.1 1.5-30	15.9±13.7 2.3-66.5	**14.5±12.1^{ac} 1.5-66.5**	<0.001 <0.001; <0.01
†Insulin Resistance (Min-Max)	1.9±0.5 0.8-2.6	2.1±0.41 1.3-2.5	**2.1±0.4^{a} 0.8-2.6**	4.8 ± 4.0 0.3-15.4	2.4 ± 2.32 0.2-6.5	**3.7±3.5^{b} 0.2-15.4**	2.5±1.9 0.4-6.4	3.3±2.6 0.4-12.4	**2.9±2.4^{c} 0.4-12.4**	<0.001 0.003; 0.02
†Plasma Resistin (ng/mL)	5.1±2.8 2.3-11.4	5.9±3.0 0.5-11.4	**5.6 ±2.9^{a} 0.5-11.4**	6.6 ±3.3 0.5-11.4	6.5±4.5 0.5-11.4	**6.6 ±3.8^{b} 2.5-18.8**	7.2±4.6 0.5-18.8	6.4±4.2 2.0-16.6	**6.7±4.2^{c} 1.85-18.5**	<0.001

Data expressed as Mean ± SD and Minimum-Maximum Values. Difference between Total Normal Weight, Overweight and Obese Parameters by One way Analysis of Variance (ANOVA) with Post hoc Bonferroni test. †Kruskal Wallis test with Pairwise Comparison for Non-Parametric Data. $^{a-c}$ Mean Ranks in a row without a common superscript letter differs**.** $^{a-a}$ Mean Ranks in a row with a common superscript letter do not differ. Level of Significance at $p \leq 0.05$. NS: Non-significant at $p > 0.05$.

Data expressed as Mean ± SD and Minimum-Maximum Values. Difference between Total Normal Weight, Overweight and Obese Parameters by One way Analysis of Variance (ANOVA) with Post hoc Bonferroni test. †Kruskal Wallis test with Pairwise Comparison for Non-Parametric Data. $^{a-c}$ Mean Ranks in a row without a common superscript letter differs. $^{a-a}$ Mean Ranks in a row with a common superscript letter do not differ. Level of Significance at $p \leq 0.05$. NS: Non-significant at $p > 0.05$.

Resistin Levels across WHO classification of Obesity in Comparison to Non-Obese Subjects

Importantly, plasma resistin levels was highest in subjects with severe obesity than non-obese

(*p<0.001)* as well as mild and moderately obese non-diabetes subjects (*p<0.001,* One-way Kruskall Wallis test with Pairwise Comparison) (Figure 2). Likewise, plasma resistin levels was higher in subjects with mild obesity than non-obese subjects. There was however, no significant difference (*p>0.05)* in resistin levels between the moderately obese and mildly obese subjects as well as moderately obese and non-obese subjects respectively (Figure 2).

Incidental Finding of Undiagnosed Hypertension and Resistin Levels

Hypertension, BP ≥ 140/90 mmHg was observed in one subject within the normal BMI category with resistin level of 11.4 µU/mL; whereas four (4) subjects in the overweight category had BP ≥ 140/90 mmHg with resistin levels ranging from 5.3 - 11.4 µU/mL. There were eleven (11) subjects in the obese category found to have hypertension with plasma resistin levels ranging between 1.85 - 14.3 µU/mL (Data not shown).

Reisistin in Relation to Selected Cardiometabolic Parameters

The study showed no significant correlation of plasma resistin with age, central obesity by waist circumference, general obesity by BMI, FBG, fasting insulin, HOMA-IR, systolic and diastolic blood pressures in all subjects by Spearman Correlation Analysis (Table 3). The same trend was found for males and female respectively (Data not shown).

When obese subjects were considered independently, there was no significant (r = 0.06; *p = 0.73)* correlation of diastolic blood pressure with resistin levels as well as with systolic blood pressure (r = 0.09; *p = 0.84)* (Data not shown).

Table 3
Relationship between Plasma Resistin and Selected Cardiometabolic Parameters by Gender in all Subjects

	MALE		FEMALE		TOTAL	
PARAMETERS	r	P-Value	r	P-Value	r	P-Value
Age (Years)	0.06	NS	0.09	NS	0.06	NS
Waist Circumference (WC)	0.15	NS	0.18	NS	0.16	NS
Body Mass Index (BMI)	0.13	NS	0.05	NS	0.08	NS
Fasting Blood Glucose (FBG)	0.07	NS	0.02	NS	0.007	NS
Fasting Insulin	-0.07	NS	-0.02	NS	0.02	NS
HOMA-Insulin Resistance (IR)	-0.03	NS	0.03	NS	-0.04	NS
Systolic Blood Pressure (mmHg)	0.13	NS	0.16	NS	0.14	NS
Diastolic Blood Pressure (mmHg)	0.14	NS	0.12	NS	0.13	NS

Spearman's Correlation Analysis. r: Correlation Coefficient.NS: Non-significant at *p > 0.05.*

Relationship of Metabolic Parameters with Obesity Indices

HOMA-IR showed no significant positive correlation with obesity indices, WC and BMI in all subjects (Table 4). However, in females subjects, HOMA-IR trended towards a marginal (*p = 0.06)* correlation with WC (r = 0.28) but not BMI (*p>0.05)*.. Similarly, HOMA-IR trended towards marginal positive correlations with WC in males (r = 0.39, *p = 0.06)*..

HOMA-IR also showed significant (*p<0.001)* positive correlation with fasting insulin (Table 4). Likewise, HOMA-IR showed significant (*p<0.0001)* correlations with fasting insulin (r = 0.96) in females with a similar trend in males (r = 0.37) though insignificant (*p<0.08)* (Data not shown).

The BMI showed significant (*p<0.001)* positive correlation with WC in all subjects (Table 4) with similar findings in females (WC, r = 0.81, *p < 0.0001)* and males (r = 0.69, p<0.0001) (Data not shown).

FBG was significantly (*p< 0.001)* negatively correlated with fasting insulin in all subjects (Table 4) with a marginal (p = 0.06) correlation in females (r = - 0.05) and no significant correlation in males (Data not shown).

Table 4
Relationship between Obesity Indices, Fasting Blood Glucose and Metabolic Parameters in all Subjects

PARAMETERS	Fasting Insulin (FI)	HOMA-IR	BMI
Waist Circumference (WC)	r = 0.11, p = 0.31	r = 0.14, p = 0.20	r = 0.78, p < 0.001*
Body Mass Index (BMI)	-0.02, p = 0.88	r = 0.02, p = 0.84	----------
Fasting Blood Glucose (FBG)	r = - 0.28, p < 0.001*	r = 0.01, p = 0.90	r = 0.19, p = 0.24
HOMA-Insulin Resistance	r = 0.93, p < 0.001*	--------	r = -0.02, p = 0.84

Spearman's Correlation Analysis. r : Correlation Coefficient; HOMA: Homeostasis Model Assessment; IR: Insulin Resistance; BMI: Body Mass Index**.** Level of Significance

Discussion

To the author's best knowledge, this is the first Nigerian study to report on resistin levels in Non-diabetes Nigerian-African adults. Preliminary animal studies showed that resistin is upregulated in rodent models of obesity and is associated with insulin resistance with its down-regulation by insulin sensitizer, rosiglitazone.[1] Later studies however, documented contrary findings attributing the lower levels of resistin in obese rodents to its suppression by free fatty acids.[28] The present study showed that in Nigerian-Africans, plasma resistin levels rose with increasing BMI categories with highest levels in subjects with severe obesity, however, with no significant positive correlation with age, insulin resistance, fasting insulin, fasting blood glucose, body mass index and central obesity. This is consistent with some previous study, which showed significantly higher resistin concentrations in overweight and obese subjects but without

correlation with insulin resistance, blood glucose, lipids, insulin and changes in body mass index & visceral fat except for positive correlations with age.[29] Contrary reports in humans however exist, in which resistin showed positive correlation with insulin resistance in type 2 diabetes, non-diabetes,[30] obese as well as healthy individuals.[1,8,9] Zaidi *et al.* showed that Pakistani individuals with severe IR had higher resistin levels than those with normal insulin action and also demonstrated a positive correlation of resistin with IR in obese non-diabetes subjects similar to diabetes subjects.[8] Some other study showed positive relations of resistin to metabolic markers such as fat mass, high density lipoprotein cholesterol (HDL-c), triglycerides, C-reactive protein and blood pressure in morbidly obese Spanish subjects without correlation to IR.[16]

On a further note, HOMA-IR were higher in overweight and obese than normal weight subjects and showed significantly (*p<0.001)* positive correlation to fasting insulin in all subjects, males and females respectively as well as, marginal correlation to central obesity majorly in females thereby, supporting the myriads of existing evidence implicating insulin resistance as an underlying mechanism in visceral obesity.[1,6–9,16] However, despite this, IR was not shown to correlate with resistin concentrations in this study similar to some other study.[31] The possible explanation for this lack of correlation may be the indirect effect of many other inflammatory markers released from the adipocytes which affect insulin resistance,[16] as well as, the fact that resistin may not be the primary determinant of IR; even though this study might be underpowered to make such an inference, as these inflammatory markers were not objectively assayed.[8,11–13,16] Recent meta-analysis has shown mild correlation of resistin with insulin resistance (IR, r = 0.21) however, with significant heterogeneity as well as increased resistin levels correlating with inflammatory markers.[11,12] Underlying mechanisms adduced are not exactly clear in humans but may be attributed to the release of pro-inflammatory cytokines inclusive of TNF-alpha and IL–6 through the nuclear factor-kB (NF-kB, p50/p65) signalling pathway,[1] on account of the effect of resistin on endothelial cells at all levels and its high expression in mononuclear cells.[5] This has been supported by both in vitro and human studies.[1–5,7–9] Resistin has several functional receptors inclusive of Toll-like receptor four (TLR–4), an isoform of decorin, receptor tyrosine kinase like orphan receptor one (ROR–1) and adenyl cyclase associated protein one (CAP–1) in both animals and humans.[1,11,17] Its binding to these sites is the first step in its activation and subsequent cascade of events that result in low grade inflammation.[16,17]

Some other reason for the lack of correlation with IR may be the lower mean resistin levels across the different BMI categories which fell within the normal range of values for the United States and European countries.[16] Zaidi et al. suggested that resistin causes insulin resistance when insulin reaches a certain critical level and resistin causes IR in hyperresistinaemic individuals as against normal resistin subjects.[8] Moreover, there are several single nucleotide genetic polymorphisms (SNPs) of the resistin RETN gene reported to be associated with resistin concentrations, but while this association remains controversial in various ethnicities, this may possibly explain the conflicting correlations reported in several studies.[1,9,32] Genetic assay was not done in this study hence cannot explain this and further studies in this direction are required in African blacks.

Consistent with a previous report, fasting insulin levels were higher in overweight and obese subjects than normal non-diabetes subjects with similar trend in resistin levels.[9] Reports have shown that resistin concentrations rise in response to supra-physiological doses of insulin in obese subjects with consequent acute regulation of resistin by insulin,[30] suggesting that subjects with higher serum fasting insulin will have higher resistin concentrations.[9] However, resistin showed no correlation with fasting insulin in this study possibly due to the influence of genetic variation[9,32] which will need to be determined in further studies.

Furthermore, age[29,30] and gender [9,32] are factors shown to influence resistin levels; however, age in this study could not be implicated as a confounder as there was no significant age difference between the three BMI categories and age showed no correlation with resistin levels. There were also no significant differences in resistin levels between the male and female subjects although it trended towards higher levels in males in all BMI categories similar to previous reports.[9,33] Another human study, on the other hand, has documented higher resistin levels in females than males,[30,34–35] while some other in rats, reported higher levels in males than females.[1,16]

Limitations

Cross-sectional studies such as this may not elucidate mechanisms or determine the cause- effect relationship of resistin on obesity in non-diabetes healthy individuals hence longitudinal/prospective[9,31] and population based studies to incorporate larger sample sizes, are advocated across the geopolitical zones of Nigeria and other sub-Saharan African countries to further clarify the role of resistin in obesity and IR. Further studies should incorporate genetic studies for the RETN gene polymorphisms[32] in African blacks as well as pro-inflammatory cytokines like TNF-alpha, IL–6 in view of their role in stimulating resistin expression and other hormones like leptin and adiponectin; as found in the white population, which may have been important confounders that should be subsequently clarified (Currently concluded - Unpublished Data).

Conclusion

Higher resistin levels were found with increasing severity of obesity in healthy non-diabetes Nigerian-Africans. HOMA-IR and fasting insulin were also higher in overweight and obese non-diabetes than normal weight subjects. However, plasma resistin showed no correlation with IR and obesity indices amongst the subjects as well as gender, suggesting possible interplay of other underlying pro-inflammatory cytokines or hormones.

Declaration

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Author Contribution

All authors contributed to the data analysis, drafting, and revision of the article as well as final approval of the version to be published. They agree to be accountable for all aspects of the work.

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Conflict of Interest
None Declared.

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Figures

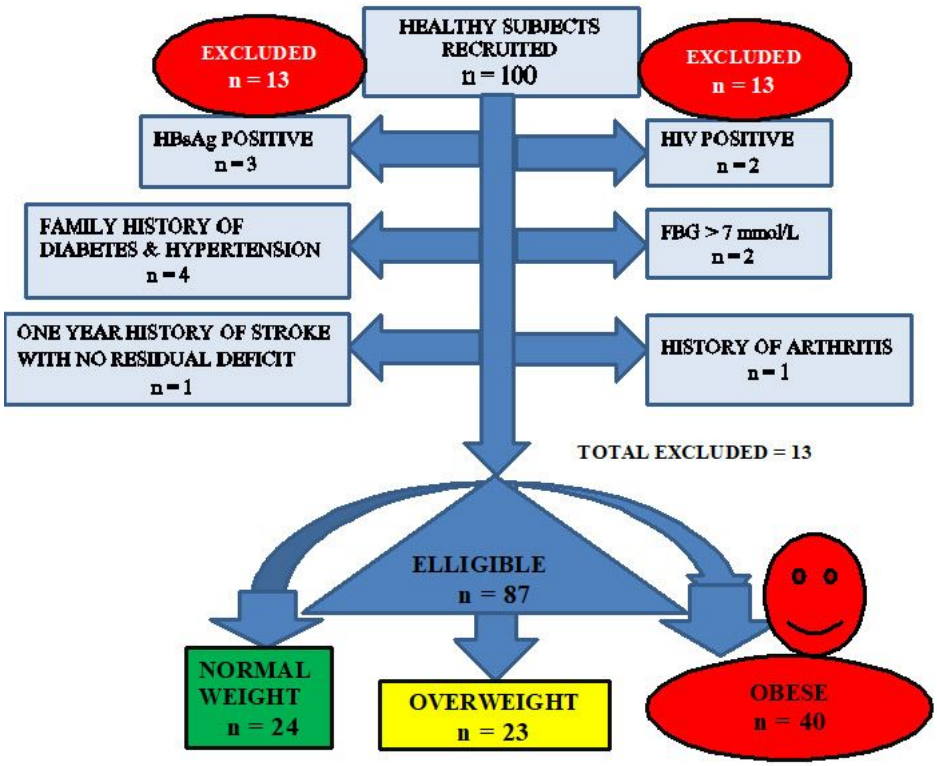

Figure 1

Study Participation of Healthy Non-Diabetes Subjects in the AdipoResistin Survey 1

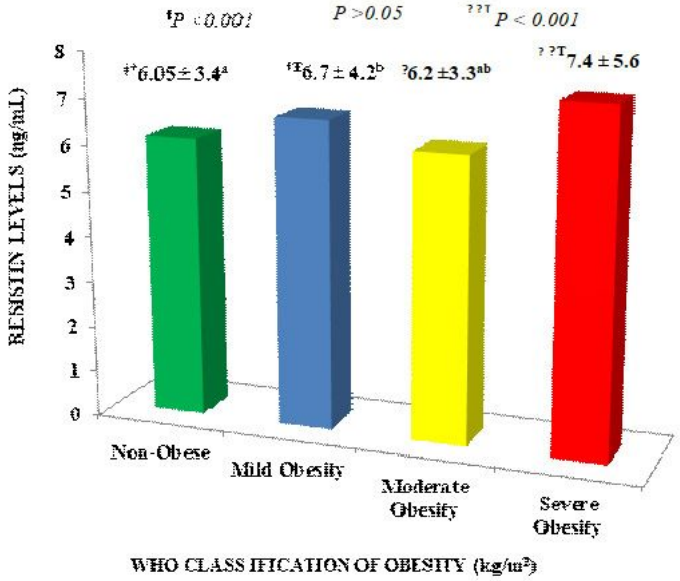

Figure 2

Plasma Resistin Levels in Non-Obese Non-diabetes Subjects in Comparison to World Health Organisation (WHO) Classes of Obesity. Analysis by One- way Kruskall Wallis test with Pair Wise Comparison. ▯ ▯ ₸ Level of significance at p ≤ 0.05. a-a, b-b Mean Ranks with a common superscript letter do no differ.