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Fasting plasma insulin in normal-weight apparently healthy adults in a Nigerian population: Reference intervals and influence of age, gender and anthropometric variables

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Abstract

Introduction: In Nigeria, there is paucity of data regarding indigenously established reference intervals for fasting plasma or serum insulin concentrations in the local populations. This study was designed to establish the reference interval of fasting plasma insulin (FPI) among apparently healthy young and middle-aged adults in a Nigerian population. The influence of age, gender, and anthropometric variables on FPI concentration were examined.

Materials and Methods: The study was a cross-sectional descriptive study involving 210 reference individuals aged 18 to 64 years. Physical, anthropometric and biochemical variables were measured including FPI and fasting plasma glucose (FPG). The reference interval for FPI was determined using the non-parametric percentile method. Correlation studies between FPI and age, anthropometric indices, and HOMA-IR were carried out.

Results: The study involved a total of 210 healthy normal-weight non-diabetic adults consisting of 110 males (52.4%) and 100 females (47.6%) as reference individuals. Reference intervals for FPI for the male, female, and total study participants were 0.1 –11.1mIU/L, 1.3 –13.2mIU/L and 0.1 – 13.03mIU/L respectively. There were statistically significant positive correlations between FPI and age (r = 0.001), WC (r = 0.0.302, p = 0.0001), WHR (r = 0.220, p = 0.0001), SBP (r = 0.137, p = 0.047) FPG (r = 0.165, p = 0.017) and HOMA-IR (r = 0.985, p = 0.0001).

Conclusion: The reference interval of FPI using the Biointecho human insulin ELISA kit is 0.1–13.0mIU/L. This is not much at variance with 0.7–9.0mIU/L quoted by the kit manufacturer, but valued widely with that of other commercially available insulin assay kits from different manufacturers. Similar to reports of similar studies, FPI correlated positively with FPG, BMI. WC, BP and age.

Keywords: Insulin; Fasting plasma insulin; Reference interval; Healthy adults; Nigeria

1. Introduction

Insulin is a 58KDa protein hormone that is synthesized and secreted by the beta-cells of endocrine pancreas[1]. It is the principal hormone that regulates glucose metabolism and homeostasis and also participates actively in the regulation of intermediary metabolism of lipids, amino acids, and proteins[2]. Circulating insulin concentration can be measured in

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the clinical laboratory for diagnostic and monitoring purposes. The potential indications for measurement of plasma or serum insulin concentrations in clinical practice include: investigation of hypoglycaemia; assessment of residual pancreatic beta-cell function especially in patients with newly diagnosed type 1 diabetes mellitus, diagnosis of insulin-secreting tumours e.g., insulinoma, differentiation of type 1 and type 2 diabetes mellitus, dynamic function tests for assessment of insulin sensitivity, estimation of some surrogate measures of insulin resistance[3-6].

In contemporary clinical practice, plasma or serum insulin assays are commonly carried out for the assessment of insulin resistance using various direct and indirect measures of insulin resistance[4,5]. In the past, measurement of fasting plasma or serum insulin concentration was considered as the most practical way of assessing for the presence of insulin resistance[6,7]. Several studies have reported strong correlation between fasting insulin concentration and insulin resistance. Thus, in healthy individuals, elevated fasting plasma insulin (FPI) levels in the presence of normal fasting plasma glucose (FPG) level, is strongly suggestive of the presence of insulin resistance[8,9]. Studies have shown that elevated FPI concentrations: (1) are associated with the presence of metabolic syndrome, (2) may predict future incidence of metabolic syndrome, (3) may presage the development of type 2 diabetes mellitus, (4) may predict future susceptibility to a number of cardiometabolic risk factors[9-13].

Despite the ongoing efforts by professional and scientific bodies, insulin assays are yet to be universally standardized[14]. For this reasons, measured insulin concentrations tend to vary according to: (1) technique and specificity of the assay method, (2) clinical laboratory, (3) local population[3,15]. Based on the above, it has been recommended that each laboratory should establish local reference intervals for plasma/serum insulin in healthy and pathological states[3,16]. In Nigeria, there is paucity of data regarding indigenously established reference intervals for fasting plasma or serum insulin concentrations in the local population. For this reason, this study was designed to establish the reference interval for FPI among apparently healthy young and middle-age adults in a Nigerian population. In addition, the study examined the influence of age, gender, and anthropometric variables on FPI concentration among the study participants.

2. Material and methods

This study was executed at the metabolic clinic of the Department of Chemical Pathology University of Calabar Teaching Hospital (UCTH), Calabar, South-South, Nigeria. The study design was cross-sectional descriptive while purposive sampling technique was employed for recruiting the reference individuals. The study participants included healthy young and middle-aged adults between the ages of 18 and 64 years. Study participants who had normal body mass index (BMI: 18.5 to 24.9 kg/m2), waist circumference (WC: <88 cm for females, <102 cm for males), glycaemia (fasting plasma glucose, FPG <6.1 mmol/L) and HbA1C <6.5%) were recruited as reference individuals. Prospective participants with clinical evidence of heart disease, kidney disease, liver disease, regular medications, chronic alcoholism and cigarette smoking were excluded from the study. A minimum sample size of 210 reference individuals (110 males and 100 females) was used for the study.

Qualitative data collection was carried out using an interviewer-administered questionnaire after a 12-hour overnight fast by the study participant. Using standard procedures, the blood pressure and anthropometric measurements including bodyweight (W), height (H) waist circumference (WC) hip circumference (HC) were measured for each study participant. Thereafter, the body mass index (BMI = Weight/Height2) and Waist-to-hip ratio (WHR = WC/HC) were estimated and expressed as kg/m2 and as a ratio respectively.

Five milliliters (5 mL) of venous blood were collected from each study participant after overnight fasting between 7:00 am and 10:00 am. 2.5 mL of collected blood each, was transferred to fluoride oxalate bottle (for glucose measurement) and lithium heparin bottle (for fasting plasma insulin measurement). After 30 minutes, both specimens were centrifuged at 3000 rpm for 10 minutes. Thereafter, the supernatant oxalated plasma and heparinized plasma were separated and transferred to storage bottles. Plasma FPG analysis was carried out within 4hours after harvesting the plasma samples. The supernatant heparinized plasma samples were stored for a maximum period of two weeks at -20 ^{o}C prior to batch analysis. Fasting plasma glucose (FPG) concentration was measured using a standard glucose oxidase method produced by Biolabo® (Biolabo SA, 02160, Maizy, France). Fasting plasma insulin (FPI) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit produced by BiO Inteco (R) (Inteco Diagnostics UK. Ltd). Procedures for both tests were carried out as recommended by kit manufactures.

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the formula: [6]

$$\text{HOMA} - \text{IR} = \frac{\text{FPI (mIU/L) x FPG (mmol/L)}}{22.5}$$

2.1. Statistical Analyses

Normality or non-normality of data was tested using the Shapiro-wilk test. Normally distributed quantitative variables were expressed as mean ± standard deviation (mean ± SD) while non-normally distributed data were expressed as median (interquartile range, IQR). The lower and upper reference limits (LRL and URL) were determined using the 2.5th and 97.5th percentile values of the data distribution respectively. Optimal threshold values of FPI were determined using the 75th and 90th percentile values. Non-normally distributed quantitative variables were compared using the non-parametric Mann-Whitney U test while parametric quantitative variables were compared using the Kruskal-Wallis test. Spearman's correlation analysis was used for the determination of relationship between non-parametric quantitative variables. A p-value of <0.05 was considered to be statistically significant. The statistical package "Statistica" (Statsoft Corp, Tulsa, OK) was used for all the statistical analyses.

3. Results

The study involved a total of 210 healthy normal-weight, non-diabetic adults consisting of 110 males (52.4%) and 100 females (47.6%). The background characteristics of the study participants are shown in Table 1. The median (IQR) of age for the male participants was 31.50 (26.00 – 41.75) years and that of females was 27.00 (24.0 – 35.0) years. The difference was statistically significant (p = 0.026).

Table 1 Background Characteristics of the overall study participants; total, male and female

Name	**Total (n = 210)**	**Male (n = 110)**	**Female (n = 100)**	**pvalue**
Age	30,0 (24,25-38.0)	31.5(26.0-41.75)	27.0 (24.0-35.0)	0.026**
BMI (kg/m2)	23.0 ±1.69	22,71 ±1.84	23.33 ±1.44	0.007**
Waist circumference	78.26 ±6.36	78.63 ±7.21	77.85 ± 5.28	0.378
Waist height ratio	0.8 (0.8-0.9)	0.8 (0.8-0.9)	0.8 (0.7-0.9)	0.0**
Systolic BP (mmHg)	115.49 ±10.53	115.56 ±9.56	115.41 ±11.56	0.916
Diastolic BP (mmHg)	70.0 (60.0-80.0)	70.0 (70.0-80.0)	70.0 (60.0-80.0)	0.042**
FPG (mmol/L)	4.5 (4.0-4.8)	4.5 (4.0-5.07)	4.3 (4.0-4.8)	0.022**
HOMA-IR	0.68 (0.32-1.28)	0.66 (0.27-1.19)	0.84 (0.46-1.66)	0.019**

** indicates statistically significant values with 0.0 meaning 0.000

A histogram representing the distribution of the FPI reference values from all the study participants showed a non-Gaussian (non-parametric) distribution (figure 1). Thus, the non-parametric percentile method was applied for the statistical determination of the LRL and URL. Using the basic bootstrap method, the 2.5th and 97.5th reference intervals and their corresponding 95% confidence limits were 0.10 (0.1 – 0.1) mIU/L and 11.72 (10.1 -16.3) mIU/L respectively.

Table 2 shows the 2.5th, 25th, 50th (median), 75th, 90th and 97.5th percentile values of the FPI in male, females and total study participants. The 2.5th , 25th, 50th (median), 75th, 90th and 97.5th percentile values of FPI for males were 0.010, mIU/L , 1.40 mIU/L , 3.15 mIU/L , 5.8 mIU/L, 10.0 mIU/L and 11.1 mIU/L ; females were 1.30 mIU/L, 2.20 mIU/L, 4.0 mIU/L, 8.45 mIU/L, 11.22 mIU/L, 13.2 mIU/L and total were 0.1 mIU/L, 1.8 mIU/L, 3.45 mIU/L, 7.0mlU/L, 10.6 mIU/L, and 13.03 mIU/L. By convention respectively, the LRL and URL correspond to the 2.5th and 97.5th percentile values. Thus, the reference intervals of FPI for male, female and total study participants were 0.1 – 11.1 mIU/L, 1.3 – 13.2 mIU/L and 0.1 – 13.03 mIU/L respectively. Also, by convention the optimal threshold value for FPI may be estimated using the 90th percentile value or the 75th percentile value of the distribution. Thus, the 90th percentile optimal threshold values of FPI for male, female, and total study participants were 10.00mIU/L, 11.22 mIU/L and 10.6 mIU/L respectively. Similarly, the 75th percentile optimal threshold values of FPI for male, female and total study participants were 5.8 mIU/L, 8.45 mIU/L and 7.0 mIU/L respectively.

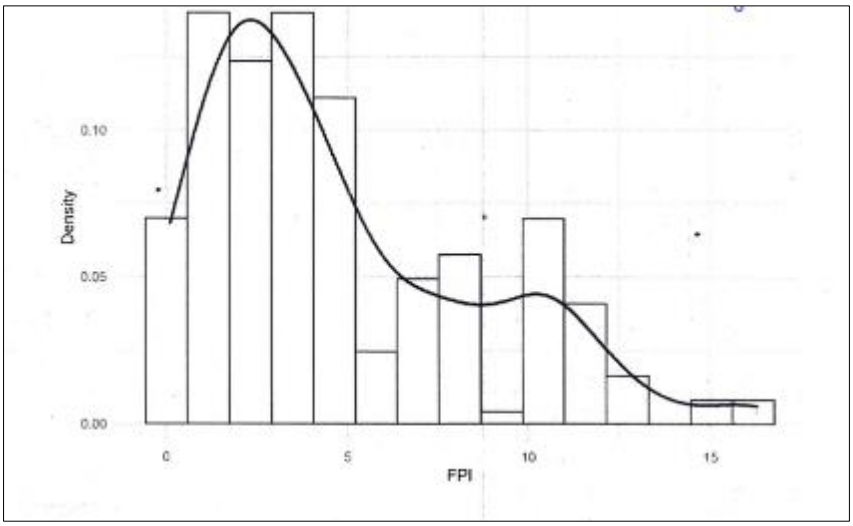

Figure 1 Frequency histogram for FPI distribution

Table 2 2.5th 25th, 50th, 75th, 90th and 97.5th Percentile FPI Values for Total, Male and Female Study Participants

		2.5th	25th	50th	75th	90th	97.5th
Total	FPI	0.1	1.8	3.45	7.0	10.6	13.03
Male	FPI	0.1	1.4	3.15	5.8	10	11.1
Female	FPI	1.3	2.2	4	8.45	11.22	13.2

Table 3 shows the correlations between FPI and the anthropometric, physical and metabolic parameters. There were statistically significant positive correlations between FPI and: age (r = 0.284, p = 0.0001), BMI (r = 0.227, p = 0.0001), WC (r = 0.302, p = 0.0001), WHR (r = 0.220, p = 0.001), SBP (r = 0.137, p = 0.047), and FPG (r = 0.165, p = 0.017). Figures 2, 3, 4 and 5 show the scatter diagrams illustrating linear correlations between FPI and BMI, WC, age, and HOMA-IR.

Table 3 Correlation between FPI and Anthropometric and Biochemical Parameters

Name	FPI	p-value
AGE	0.284	0.0001
BMI	0.227	0.001
WC	0.302	0.0001
WHR	0.22	0.001
SBP	0.137	0.047
DBP	-0.035	0.616
FPG_mmol/L	0.165	0.017
HOMA-IR	0.985	0.0001

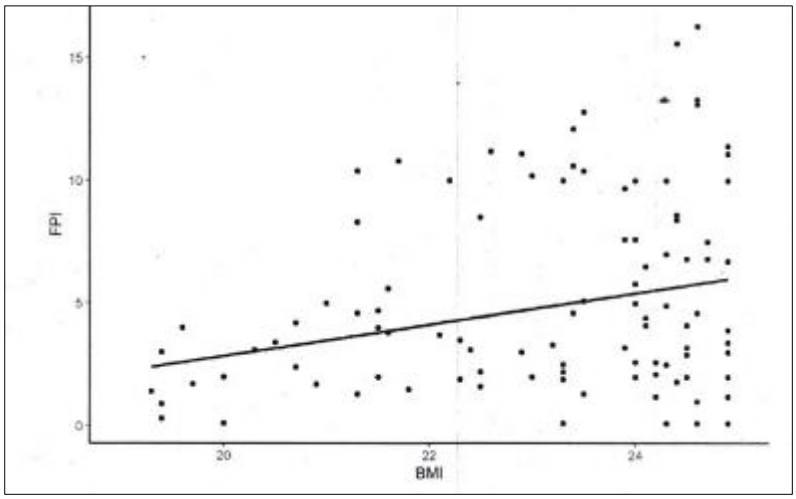

Figure 2 Scatter plot showing correlation between FPI and BMI

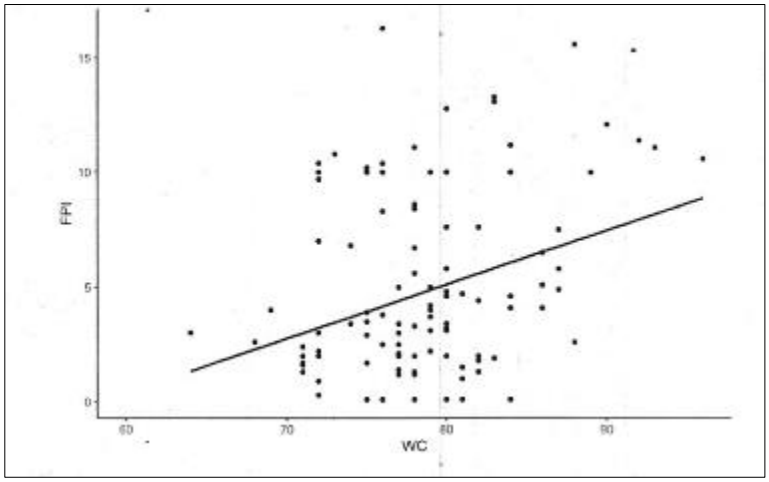

Figure 3 Scatter plot showing correlation between FPI and WC

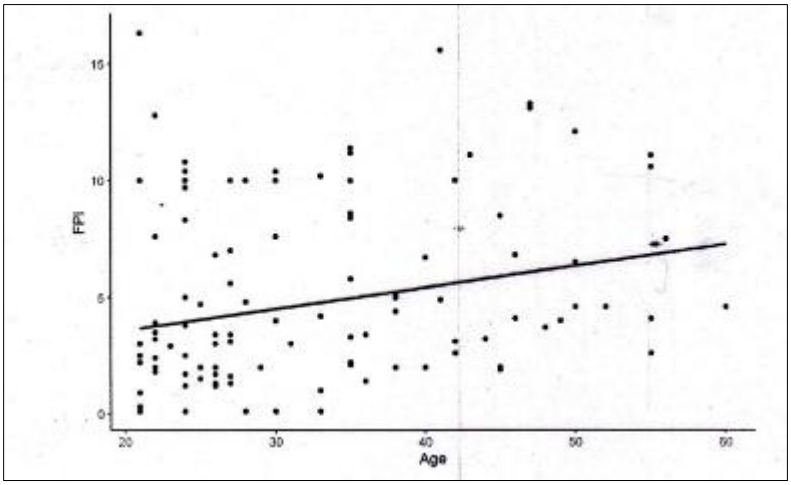

Figure 4 Scatter plot showing correlation between FPI and age

4. Discussion

This present study was designed to establish a local health-associated reference interval for FPI in a Nigerian adult population. Measurement of FPI or fasting serum insulin (FSI) concentration is one of the simplest, cheap, and less-invasive ways of assessing the presence of insulin resistance in contemporary clinical practice.6,7 In addition, other potential indications for measurement of FPI in the clinical laboratory include: diagnosis of the presence of insulin-secreting tumours, differentiation between type1 DM and type 2 DM, evaluation of patients with hypoglycaemia, and assessment of residual pancreatic β-cell function.7 For measured FPI to be used for the above indications, there is the need to compare the measured FPI concentration with normative FPI values established from apparently healthy reference individuals within the local population that the laboratory serves.17 Furthermore, because of lack of the universal standardization and harmonization of insulin immunoassay methods, it has been recommended that each clinical laboratory should establish its local normative values for FPI using healthy individuals among the indigenous population that it serves.14 This in particular was the main reason behind the conceptualization and execution of this study.

From this study, the central 95% reference intervals for the male, female, and total study participants were 0.1–11.1mIU/L, 1.3 – 13.2mIU/L and 0.1 – 13.0mIU/L respectively. These values are by virtue of their magnitudes similar to the reference interval quoted by the assay kit manufacturer (Biolnteco®, United Kingdom), that is, 0.7 – 9.0mIU/L. In contemporary clinical laboratory practice in Nigeria, several commercially available immunoassay kits for the measurement of FPI abound. Prominent among these immunoassay kits which are based on the enzyme-linked immunosorbent assay (ELISA) technique include those produced by fortress Diagnostics®, Calbiotech®, Diagnostic Automation®, Elabscience®, Accubind®, and BioInteco®. Among these in vitro diagnostic kits, the one manufactured by BioInteco® has attained commonplace use by the majority of clinical laboratories in Nigeria. This justified the establishment of reference intervals for FPI using this comparatively common immunoassay kit for measurement of plasma or serum human insulin level.14

As mentioned previously, this study obtained the reference interval of FPI as 0.1 – 13.0 mIU/L for the total study participants, while the manufacturer's normative value is 0.7 – 9.0mIU/L. A review of the common immunoassay kits for measurement of FPI concentration revealed the reference intervals of FPI 0.0 – 30.0mIU/L; 0.78 – 50.00mIU/L; 4.7 – 30.0mIU/L; and 0.2 – 2.5mIU/L, 5 – 35Miu/L for Fortress Diagnostics® Elabscience®, Abcam Diagnostics®, DRG®, and Diagnostic Automation respectively. In addition, a Nigeria study in Zaria, Kaduna State, using the DRG® insulin ELISA kit reported a normative value of 0.356 – 0.788mIU/L for FPI in healthy adults.18 The review above showed a wide variation of reference intervals of FPI using the various insulin ELISA Kits. The reason for these differences is not far-fetched. First, the different insulin immunoassay kit manufacturers use different insulin antigens to produce the monoclonal antibodies used in the production of the assay kits.15,16 Secondly, the insulin assay by the ELISA method is yet to be standardized and harmonized globally. Thus, different assay kit manufacturers only quote the reference values that they established using their locally produced immunoassay kits.14 Thirdly, the reference interval values vary according to the different populations used for their determinations.16 Based on the above variations, it has been strongly recommended that each local clinical laboratory, before using any of the above insulin assays for diagnostic or monitoring purposes, should endeavour to establish an indigenous reference interval for the local population that it serves.18,19 This, for now, should be the acceptance practice until the universal standardization of insulin immunoassay methods is achieved.

Fasting plasma insulin level has been shown by several studies to be a fairly good surrogate marker of insulin resistance especially in healthy non-diabetic individuals.6 This present study revealed a statistically significant positive correlation between FPI and HOMA-IR which is a common surrogate measure of insulin resistance in contemporary any clinical practice. This finding is consistent with previous studies that demonstrated considerable correlation between FPI and measures of insulin resistance such as HOMA-IR and hyperinsulinaemic euglycaemic glucose clamp (HEGC).6,20 Essentially, increased FPI levels in the presence of normal FPG level is highly suggestive of the presence of insulin resistance.6 It is worthy of note that the linear relationship between FPI and insulin resistance was mostly demonstrated among non-diabetic individuals. In contrast, a substantial overlap in FPI levels has been demonstrated between healthy non-diabetic subjects and those with established insulin resistance states such as prediabetes and T2DM.21

Type 2 diabetes mellitus may be associated with an appropriately high normal or low FPI levels. Thus, FPI concentration may not be a reliable marker of insulin resistance among patient with some insulin resistance state's such as T2DM.21 For instance, ter Horst et al recently suggested that FPI can be used to reliably diagnose insulin resistance in obese non-diabetic individuals.8 Overall, the use of FPI as a surrogate marker of insulin resistance is limited by high levels of false positive results as well as lack of standardization of insulin immunoassay techniques.14,21

Our study showed a statistically significant linear relationship between age of study participants and FPI. This finding has been corroborated by a good number of studies especially among non-diabetic healthy individuals. Atria et al reported a direct correlation between proinsulin (a precursor of insulin) and age among adults in the general population.22 Insulin resistance is generally known to increase with age. In most cases, the phenomenon of insulin resistance is accompanied by compensatory insulin hypersecretion by β-cell of pancreas with consequent hyperinsulinaemia.7 Thus, the older an individual becomes, the more likely he or she is prone to develop insulin resistance with attendant increased levels of FPI. The increased steady-state plasma insulin concentration with age especially among subjects with background obesity and insulin resistance has been attributed in part, as a potential cause of high association between aging, obesity, metabolic syndrome, and cancer.2,11

This study found the reference intervals of FPI for the female and male study participants to be 1.3 – 13.2 mIu/L and 0.1 – 11.1 mIu/L respectively. From these values, the females had slightly higher normative values than their male counterparts. Nevertheless, the difference is not statistically significant. Varying findings regarding the differential reference intervals of FPI in male and female adult populations have been reported. Whereas some studies reported slightly higher values in non-pregnant adult females, some other studies observed comparatively higher values in adult males. Nevertheless, most studies recommended the use of harmonized reference values for FPI.6

This study showed significant positive correlations between FPI and obesity-defining anthropometric variables such as BMI, WC and WHR. These findings are in line with other studies and reviews which have demonstrated proportionate association between obesity and insulin resistance.8 Presently, there is an extant argument regarding the cause-and-effect relationship between obesity and insulin resistance.22 In addition, some studies have reported a positive association between FPI and metabolic syndrome and its components such as WC and SBP. Unsurprisingly, some definitions of the metabolic syndrome involved the presence of fasting hyperinsulinaemia.11-13

5. Conclusion

In this study, we have been able to establish indigenous health-associated reference interval for FPI using a commonly used commercially available human insulin ELISA kit. The overall reference interval of 0.1 – 13.0 mIU/L obtained by this study is not much at variance with 0.7 – 9.0 mIU/L quoted by the kit manufacturer. Nevertheless, we strongly recommend the subsequent use of this locally established value by local clinical laboratories that will make use of the same ELISA kit for measurement of plasma or serum FPI. Similar to the observations by previous studies, FPI correlated with FPG, BMI, WC, BP and age. The findings of this study will help improve the use of FPI measurements in the evaluation of metabolic and cardiovascular disorders associated with alterations in insulin secretion and sensitivity.

Limitations of the study

This study has few limitations. First, the study was based on a relatively small sample size. Nevertheless, the total number of the study participants in this study (n = 210) is far greater than the standard minimum sample size (n = 120) recommended for the establishment of reference interval by the CLSI.19 The second limitation still remains the fact that the BioInteco human insulin ELISA kit used for this study was not standardized. Hence, the FPI values and the determined reference interval cannot be compared quantitatively to the values obtained by similar studies that used other human insulin ELISA kits produced by different immunoassay kit manufacturers.

Compliance with ethical standards

Disclosure of conflict of interest

Emeka Callistus Onyeka Izuchukwu, Henry Chima Okpara, Chisom Adaobi Nri-Ezedi, F I Allison, and Obianuju Uchenna Ilechukwu do not have any conflict of interest with anyone or organization with respect to the publication of this article.

Statement of ethical approval

Ethical approval for the study was obtained from the Joint Ethical Committee of College of Medicine University of Calabar/University of Calabar Teaching Hospital. Selected study participants provided informed written consent before their final enrollment in the study.

Statement of informed consent

Informed consent was obtained from all study participants included in the study.

Authors' contribution

ECOI and HCO conceptualized the work. ECOI, HCO, and CAN contributed to the literature review. HCO, ECOI, and FIA prepared the manuscript. ECOI, HCO, CAN and IOU proofread the final manuscript before submission. ECOI, HCO, FIA, and IOU assayed all samples. CAN, HCO and ECOI handled statistical analyses. All authors approved the submission of the manuscript for publication.

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