Biologic Behavior and optimal cut-off point estimation for Serum Fasting Insulin: A report from the Maracaibo City Metabolic Syndrome Prevalence Study

Comportamiento biológico y la estimación de punto de corte óptimo para suero insulina en ayunas: Un informe del estudio de prevalencia del Síndrome Metabólico de la Ciudad de Maracaibo

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Abstract

Introduction: Insulin resistance is a metabolic state in which tissue effects of insulin are diminished, leading to hyperinsulinemia as a compensatory mechanism. The combination of insulin resistance and hyperinsulinemia constitutes one of the main pathophysiological landmarks of Metabolic Syndrome. Thus, our main objective was to determine a cut-off point for serum fasting insulin in the population of Maracaibo.

Materials and Methods: Descriptive, cross-sectional study realized in 2,026 subjects of both genders, 18 years of age or older, who had their serum fasting insulin quantified. These values underwent logarithmic transformation for normalization of their distribution, which was corroborated through Geary's test. Results were expressed as means ± SD. A cut-off was selected through the construction of ROC Curves using selected healthy and "sick" populations.

Results: In the studied population (n=2,026) the mean serum fasting insulin concentration was 14.6±9.5 µU/mL. When stratifying by gender, a mean of 14.5±9.3 µU/mL was observed in women and 14.8±9.8µU/mL in men; p=0.715. When assessing these levels by age groups, BMI and waist circumference, a progressive increase was observed along each category. The selected cut-off point for serum fasting insulin concentration was 13µU/mL; AUC=0.792; Sen=74.4%; Esp=71.3%.

Conclusions: Serum fasting insulin concentrations increase with age, BMI and waist circumference. A cut-off point of 13µU/mL is suggested for the definition of fasting hyperinsulinemia in our population.

Key Words: Insulin, Hyperinsulinemia, Insulin Resistance, Metabolic Syndrome, Cut-Off point.

Introducción

Insulin resistance (IR) is a metabolic disorder in which the peripheral tissue effects of insulin are diminished, and such phenomenon is compensated through an increase in its secretion by the pancreatic β cell, generating "compensatory hyperinsulinemia"[1]. Although in initial stages, most subjects are able to maintain a progressive elevation of insulin concentrations to counterbalance this alteration, the added strain it entails on the pancreatic β cell is a double-edged sword. Indeed, notwithstanding the prevention of overt hyperglycemia by this compensatory hyperinsulinemia, it also greatly augments both short and long term risk of developing Type 2 Diabetes Mellitus (T2DM), obesity, high blood pressure (HBP) and dyslipidemia, characterized by elevated triacylglyceride (TAG) levels, and low concentrations of serum high-density lipoprotein cholesterol (HDL-C)[2].

Over 20 years ago, Reaven proposed that subjects presenting this constellation of alterations associated with IR-compensatory hyperinsulinemia may exhibit a significantly greater risk of developing Cardiovascular Disease (CVD), first outlining the Syndrome X[3].In the following decades, not only has Reaven's hypothesis been confirmed, but IR and hyperinsulinemia have also been established as the main etiopathogenic component of this syndrome, currently known as the Metabolic Syndrome (MS)[4].Although the prevalence of MS in Latin America has been estimated at 24.9% in 2011[5]; in our locality it is present in 42.4% of the population, exhibiting one of the greatest MS prevalences in Latin America[6]. In consequence, MS currently constitutes one of the main problems in public health both worldwide and nationwide.

Although MS is one of the main entities associated with hyperinsulinemia, several other pathologies have been associated with this alteration, including coronary artery disease, myocardial hypertrophy, cardiac arrhythmias, cerebrovascular disease, non-alcoholic fatty liver disease, and even some types of cancer[5, 8-11].These implications highlight the importance of IR-hyperinsulinemia, which has led to numerous studies determining cut-off points for fasting insulin concentration in diverse populations, in order to offer early detection and prevention of the related pathologies. Indeed, because genetic and environmental factors greatly modify these concentrations in diverse populations worldwide[12], specific cut-off points for each population are of utmost importance for determining accurate diagnosis and prevention of these endocrine-metabolic disorders.

This scenario, in addition to the scarcity of population studies on the epidemiologic behavior of fasting insulin in our country and Latin America, the objective of this report was to determine the cut-off point for hyperinsulinemia in adult subjects in Maracaibo, Venezuela.

Materials and Methods

Population Selection

The Maracaibo city Metabolic Syndrome Prevalence Study (MMSPS) is a cross-sectional study which took place in the city of Maracaibo-Venezuela, with the purpose of identifying and analyzing Metabolic Syndrome and Cardiovascular risk factors in the adult population of Maracaibo, the second largest city of Venezuela, with 2.500.000 inhabitants. The methodological process for the selection of the individuals to obtain a representative sample has been published elsewhere[13]. There are currently 2230 individuals enrolled, with complete physical examination, laboratory measurements and anamnesis; for this branch of the study 2,026 individuals were selected on the basis of availability of serum fasting insulin quantification.

For the determination of the cut-off point, a subsample of 554 individuals were selected (reference population), based on the following inclusion criteria (Figure 1): Absence of obesity (BMI <30 kg/m^2, Waist Circumference <93.5 cm in women, <98 cm in men), absence of MS, no personal history of HBP, Type 1DM, thyroid or hepatic disease, angor pectoris, myocardial infarction, arrhythmia or cerebrovascular diseases, or polycystic ovary syndrome, and no consumption of medication which could influence glycemia or lipid profile. Another reference "sick" population consisting of 379 subjects was selected, according to the following criteria: Presence of obesity (BMI ≥30 kg/m^2) and MS, excluding subjects with T2DM or consuming medication which could influence glycemia or lipid profile.

Subjects were evaluated using routine medical examination chart provided by the Health and Social Development Ministry of Venezuela as data collecting tool. The study was approved by the Ethics Committee of the Endocrine and Metabolic Diseases Research Center "Dr. Félix Gómez" from the University of Zulia - Venezuela, and all participants signed a written consent prior to any involvement, interrogation, physical examination and blood sampling.

Subject evaluation

The assessment of blood pressure was done using a calibrated mercury sphygmomanometer, with the subject previously rested (15 minutes at least) in a sitting position with both feet touching the floor. Two determinations were made with an interval of 15 minutes and averaged. The arm was positioned at heart level, and a proper sized cuff used for the procedure. Systolic blood pressure was determined when the first Korotkoff sound is heard, while diastolic blood pressure was determined at the fifth Korotkoff sound. Blood Pressure classification was completed using the criteria proposed in the VII Joint National committee (JNC-7)[14].Obesity was classified applying the WHO criteria[15] based on the BMI formula [Weight/Height^2, expressed in Kg/m^2]. Weight was assessed using a digital scale (Tanita, TBF-310 GS Body Composition Analyzer, Tokyo – Japan), while Height was obtained with a calibrated rod in millimeters and centimeters; the subjects were barefooted and wearing light clothing at all times. Waist Circumference (WC) was measured using calibrated measuring tape in accordance to the anatomical landmarks proposed by the USA National Institutes of Health protocol[16].

Laboratory Analysis

Fasting levels of glucose, total cholesterol, TAG, and HDL-C were determined with a computerized analyzer (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). The intra-assay variation coefficient for the total cholesterol, TAG, and HDL-C was 3%, 5%, and 5% respectively. LDL-C and VLDL levels were calculated applying Friedewald's formulas(17) only if triglycerides were <400 mg/dL; higher levels were measured using lipoprotein electrophoresis. Likewise, serum high-sensitivity C-Reactive Protein (hs-CRP) concentration was quantified employing immune-turbidimetric essays (Gesellschaft für Biochemica und Diagnostica mbH, Germany). Serum fasting insulin was determined using an ELISA double-sandwich method (DRG Instruments GmbH, Germany, Inc). For the diagnosis of MS, the criteria from the IDF/AHA/NHLBI-2009 consensus were applied[18].

Statistical Analysis

Normal distribution of continuous variables was evaluated by using Geary's test; variables without normal distribution were logarithmically transformed and reevaluated in order to corroborate successful normalization of their distributions. Results were expressed as arithmetic mean ± standard deviation. Significant differences between arithmetic means were established using Student´s "t" test (when two groups were compared) or ANOVA (when three or more groups were compared) with Tukey's test

post-hoc analysis. Qualitative variables were expressed as absolute and relative frequencies, the c^2 to assess association between variables, results were considered statistically significant when $p<0.05$.

Insulin results were expressed in percentile when deciding for a cutoff point for the hormone; otherwise, it was expressed as mean ± SD for general purposes. To further explore fasting insulin cut-offs, a Receiving Operating Characteristic method (ROC Curve) was applied using the healthy and sick reference populations (Figure 1). To establish the optimal cut-off for basal insulin several indexes were used. The Youden Index was calculated using the formula [J = sensitivity + specificity – 1 = S-(1-Es)] (19), The minimal cut-off value was calculated using the distance of the point closest to (0.1) on the ROC curve formula: square root [(1-sensitivity)^2 + (1-specificity)^2] (20). Moreover, Positive [sensitivity /1-specificity] and Negative [1-sensitivity/specificity] (21).The database analysis was done using the Statistical Package for the Social Sciences (SPSS) v. 20 for Windows (IBM Inc. Chicago, IL),), while the ROC curves were constructed using the R Project for Statistical Computing, developed at Bell Laboratories, available at http://www.r-project.org/.

Results

Characteristics of the general population

A total of 2026 individuals were studied, of which 47.9% (n=970) were males and 52.1% (n=1056) were females. The mean age of the population was 39.7±15.4 years. The general characteristics of the population are summarized in Table 1.

Fasting Insulin, Gender and Age Groups

Arithmetic mean of fasting insulin concentration for the general population was 14.6±9.6 µU/mL. When categorizing by sex(Figure 2, Panel A), women displayed an average fasting insulin level of 14.6±9.3 µU/mL, whereas men presented a mean concentration of14.8±9.8 µU/mL; p=0.715.Serum fasting insulin concentrations depict a progressive increase regarding age in the main population, up to the group of 30-39 years (Figure 2, Panel B), with statistically significant differences between subjects with 20-29 years and 30-39 years of age (15.4±9.1 µU/mL vs 14.0±9.7 µU/mL, respectively; p=0.037).

Fasting Insulin and Body Mass Index

The behavior of fasting insulin according to BMI categories in the main population is represented in Table 2, describing an ascending trend as BMI increased. The mean concentration of the Low Weight subjects was 9.7±6.2µU/mL, whereas the Obesity III category had a mean of µU/mL; $p=2.87x10^{-4}$. Likewise, fasting insulin levels also describe a similar pattern regarding BMI by gender (Figure 3), showing a progressive increase across categories in both sexes; in females, the Low Weight group had a mean of 10.6±6.8 µU/mL, while Obese Type III women had 19.8±12.3 µU/mL. Meanwhile in males, the Low Weight category displayed a mean fasting insulin level of 7.2±3.1 µU/mL, increasing up to 29.6±15.8 µU/mL in the Obese Type III group. The differences among these means can be observed in Table 3.When comparing fasting insulin means between sexes, significant differences were ascertained in the Normal Weight group, where women showed higher levels than men (12.0±6.3 µU/mL vs 10.1±6.4 µU/mL, respectively; $p=4.54x10^{-7}$); conversely, in the Obesity Type III category, fasting insulin levels were significantly greater in males (Females: 19.8±12.3 µU/mL vs Males: 29.6±15.8 µU/mL; p=0.001).

Fasting Insulin and Waist Circumference

The WC value for each Quartile in men were: Q1:<88cm, Q2: 88-96.99 cm, Q3: 97-106.99 cm, and Q4: (≥107 cm); meanwhile, women obtained the following: Q1: (< 81cm); Q2: (81-89.99 cm); Q3: (90-99.99 cm) and Q4: (≥100 cm). Table 2 depicts the behavior of serum fasting insulin concentration according to quartiles of waist circumference (WC) in the main population, where a progressive increase in fasting insulin levels was observed across WC quartiles. In the female group, the mean and standard deviation for fasting insulin in each WC quartile were as follows: Q1: 12.31±7.07 µU/mL; Q2: 13.07±7.19 µU/mL; Q3: 13.74±6.88 µU/mL; and Q4: 18.72±12.94 µU/mL. On the other hand, the men obtained the following fasting insulin results in each quartile: Q1: 9.62±5.39 µU/mL; Q2: 12.58±8.02 µU/mL; Q3: 15.61±9.49 µU/mL; and Q4: 20.98±11.34 µU/mL. The differences observed in regards to gender, were on Quartiles 1 ($p= 5.61x10^{-8}$) and 4 (p=0.001). When comparing by sex (Figure 4), a similar trend is observed in both genders; women had a mean fasting insulin level of 12.3±7.1 µU/mL in Q1, and 18.7±12.9 µU/mL in Q4. Men exhibited a fasting insulin average of 9.6±5.4 µU/mL in Q1, and µU/mL in Q4. These differences were significant within both genders. Finally, while comparing fasting insulin means between men and women, significant differences were found only in Q1 ($p=5,61x10^{-8}$); and in Q4 (p=0,001).

Fasting Insulin in the reference population and determination of cut-off point for hyperinsulinemia

The reference population was constituted by 602 subjects, 47.3% (n=285) females and52.7% (n=317) males. Table 5 summarizes the general characteristics of this population by gender. In this part of the results, insulin was expressed in percentiles in order to select a valid cut-off point. The 50th percentile for fasting insulin level in these healthy subjects was 10.2µU/mL, with a 25th percentile (p25) of 7.4µU/mL and a 75th percentile (p75) of13.6µUI/mL. When assessing the reference population by gender, the 75th percentile for fasting insulin level in women was 14.4µU/mL; while the men's 75th percentile was 12.7µU/mL (Table 6).

The determination of an appropriate cut-off point for fasting insulin was done through the construction of a ROC Curve in subjects selected through the criteria presented in Figure 1. The overall ROC curve rendered an AUC of 0.792 with a cut-off point of 13.05μU/mL (74.4% Sensitivity and 71.3% specificity). Figure 5 shows ROC curve for women with an AUC of 0.733 with a cut-off of 13.15μU/mL (71.2% Sensitivity and 66.00% specificity), while ROC for men displayed an AUC of 0.838 with a cut-off of 11.95μU/mL (82.1% Sensitivity and 72.6% specificity); Delong test shows non-significative differences between both AUC; p=0.367. Table 7 shows the results from the indexes used to determine the cut-points selected in the curves.

Table 1. General characteristics of the general population by gender

	Females (n=1056)		Males (n=970)		Total (n=2026)	
	n	%	n	%	n	%
Age Groups (Years)						
<20	87	8.2	69	7.1	156	7.7
20-29	221	20.9	294	30.3	515	25.4
30-39	178	16.9	183	18.9	361	17.8
40-49	242	22.9	180	18.6	422	20.8
≥50	328	31.1	243	25.1	571	28.2
BMI Categories						
Low Weight	29	2.7	11	1.1	40	2
Normal Weight	348	33	234	24.1	582	28.7
Overweight	333	31.5	386	39.8	719	35.5
Obesity I	208	19.7	215	22.2	423	20.9
Obesity II	92	8.7	83	8.6	175	8.6
Obesity III	46	4.4	41	4.2	87	4.3
Elevated Waist Circumference*						
Present	836	79.2	694	71.5	1530	75.5
Absent	220	20.8	276	28.5	496	24.5
Metabolic Syndrome*						
Present	422	40	444	45.8	866	42.7
Absent	634	60	526	54.2	1160	57.3
Total (%)	1056	52.1	970	47.9	2026	100

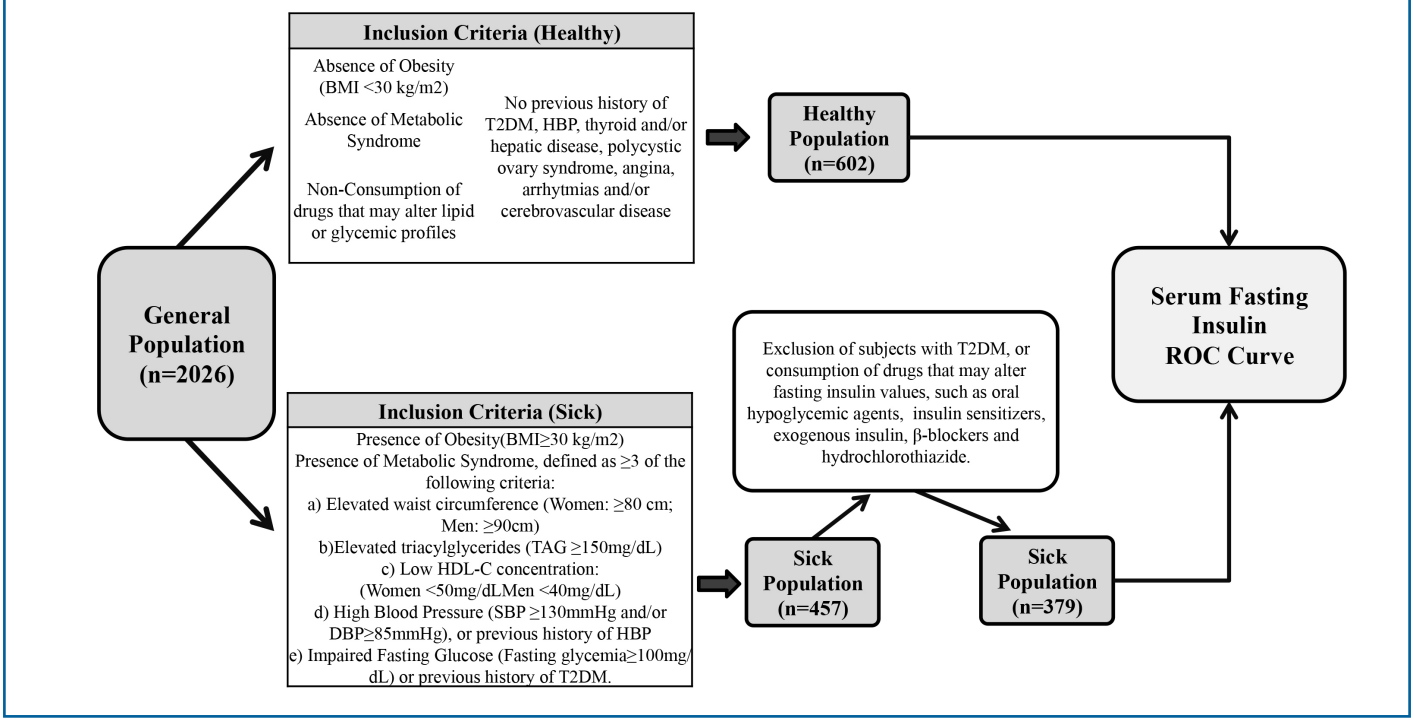
BMI=Body Mass Index

* Criteria from the IDF/AHA/NHLBI- 2009 consensus.

Table 2. Serum fasting insulin concentration in the general population by BMI categories and waist circumference quartiles.

	Mean	SD	*p**
BMI Categories			**$2.87x10^{-4}$**
Low Weight	9.6	6.1	
Normal Weight	11.2	6.4	
Overweight	13.5	7.7	
Obesity I	17.4	10.3	
Obesity II	20.4	12.6	
Obesity III	24.4	14.8	
Waist Circumference for Men			**$5.37x10^{-42}$**
Quartile 1	9.62	5.39	
Quartile 2	12.58	8.02	
Quartile 3	15.61	9.49	
Quartile 4	20.98	11.34	
Waist Circumference for Women			**$9.09x10^{-18}$**
Quartile 1	12.31	7.07	
Quartile 2	13.07	7.09	
Quartile 3	13.74	6.88	
Quartile 4	18.72	12.94	

Figure 1. Flow chart for the selection of healthy and sick reference populations for the construction of ROC Curves for the determination of serum fasting insulin cut-off values.



BMI=Body Mass Index; T2DM=Type 2 Diabetes Mellitus; HBP=High Blood Pressure; HDL-C=High-Density Lipoprotein Cholesterol; TAG=Triacylglycerides; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure.

Table 3. p values of the One-Way ANOVA test when comparing serum fasting insulin concentration by BMI categories in each gender.

Males

BMI	Low Weight	Normal Weight	Overweight	Obesity I	Obesity II	Obesity III
Low Weight	-	NS	NS	$4.59x10^{-5}$	$3.44x10^{-7}$	$4.22x10^{-5}$
Normal Weight	NS	-	NS	$1.98x10^{-10}$	$3.71x10^{-12}$	$9.39x10^{-6}$
Overweight	NS	NS	-	$1.73x10^{-5}$	$5.16x10^{-8}$	0.001
Obesity I	$4.59x10^{-5}$	$1.98x10^{-10}$	$1.73x10^{-5}$	-	NS	NS
Obesity II	$3.44x10^{-7}$	$3.71x10^{-12}$	$5.16x10^{-8}$	NS	-	NS
Obesity III	$4.22x10^{-5}$	$9.39x10^{-6}$	0.001	NS	NS	-

Females

BMI	Low Weight	Normal Weight	Overweight	Obesity I	Obesity II	Obesity III
Low Weight	-	NS	0.002	$1.03x10^{-6}$	$2.55x10^{-8}$	$4.66x10^{-13}$
Normal Weight	NS	-	$6.26x10^{-18}$	$2.73x10^{-13}$	$2.73x10^{-13}$	$2.73x10^{-13}$
Overweight	NS	$6.26x10^{-18}$	-	$2.59x10^{-8}$	$1.67x10^{-9}$	$2.73x10^{-13}$
Obesity I	$1.03x10^{-6}$	$2.73x10^{-13}$	$2.59x10^{-8}$	-	NS	$1.87x10^{-7}$
Obesity II	$2.55x10^{-8}$	$2.73x10^{-13}$	$1.67x10^{-9}$	NS	-	0.003
Obesity III	$4.66x10^{-13}$	$2.73x10^{-13}$	$2.73x10^{-13}$	$1.87x10^{-7}$	0.003	-

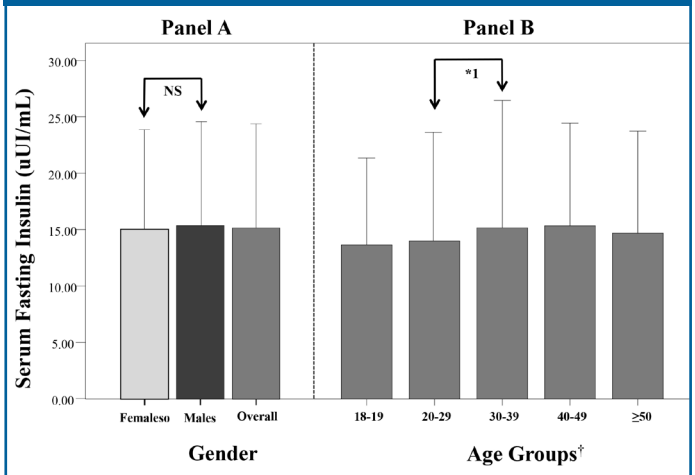
NS: Not significant.

Table 5. General characteristics of the reference healthy population by gender.

	Women (n=285; 47.3%)		Men (n=317; 52.7%)		Total (n=602; 100%)		p*
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	29.6	11.3	29.7	10.9	29.5	11.6	0.768
BMI (kg/m^2)	24.0	3.1	23.5	3.2	24.5	3.0	$2.8x10^{-5}$
WC (cm)	84.2	9.1	81.3	8.7	86.8	8.6	$2.8x10^{-14}$
Fasting glycemia (mg/dL)	87.8	9.9	88.4	9.0	87.3	10.6	0.106
Fasting Insulin (µUI/mL)	11.6	7.2	12.6	7.8	10.6	6.5	0.001
HOMA2-IR	1.7	1.0	1.8	1.0	1.6	0.9	0.001
Total Cholesterol (mg/dL)	174.0	39.2	172.0	35.5	175.9	42.3	0.431
Triacylglycerides (mg/dL)	85.7	47.5	74.4	35.3	95.9	54.3	$3.0x10^{-19}$
VLDL (mg/dL)	14.88	7.07	19.18	10.86	17.14	9.50	$1.05x10^{-8}$
LDL-C (mg/dL)	109.2	35.0	107.5	31.6	110.8	37.7	0.847
HDL-C (mg/dL)	47.6	12.6	49.7	12.0	45.8	12.9	$8.2x10^{-6}$
SBP(mmHg)	111.3	11.2	108	9.7	114.3	11.7	$4.4x10^{-12}$
DBP (mmHg)	71.8	8.8	70.0	8.2	74.0	9.0	$1.4x10^{-6}$

* t Student Test after logarithmic transformation between gender.
BMI=Body Mass Index; WC=Waist Circumference; LDL-C=Low-Density Lipoprotein Cholesterol; HDL-C= High-Density Lipoprotein Cholesterol; SBP=Sistolic blood pressure; DBP=Dyastolic blood pressure.

Figure 2. Serum fasting insulin concentration in the general population by gender and age groups.



†One-Way ANOVA: $p = 0.075$ *1. Post-hoc Tukey test: $p = 0.037$

Figure 3. Serum fasting insulin concentration in the general population by Body Mass Index categories and gender.

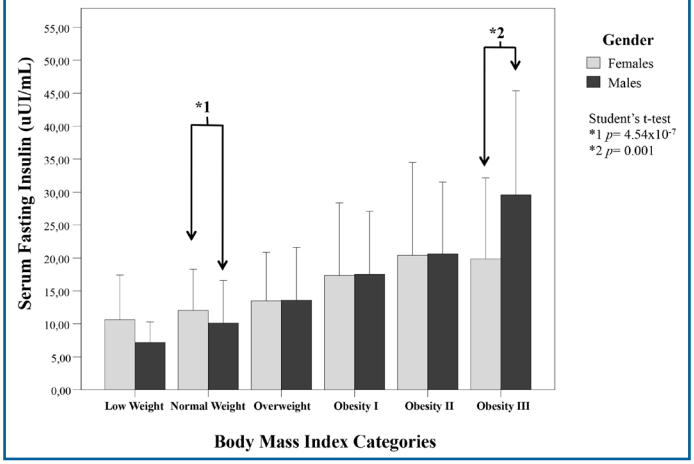
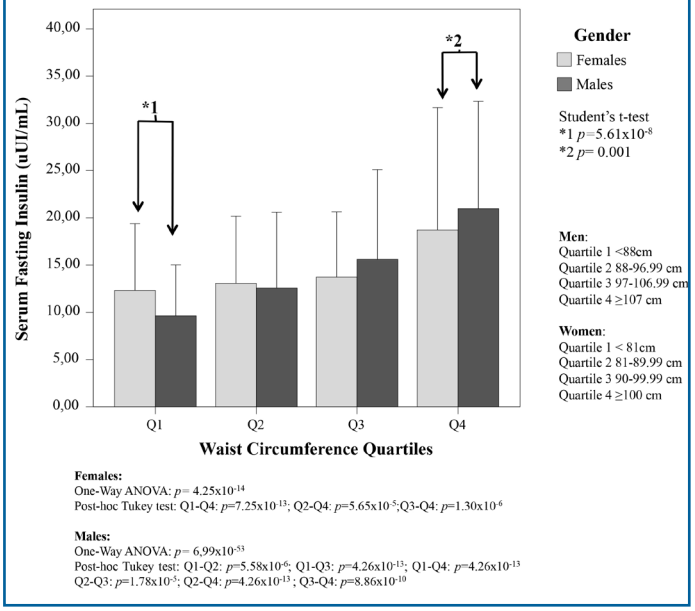


Figure 4. Serum fasting insulin concentration by waist circumference quartiles and gender.



Females:
One-Way ANOVA: $p = 4.25x10^{-14}$
Post-hoc Tukey test: Q1-Q4: $p=7.25x10^{-13}$; Q2-Q4: $p=5.65x10^{-5}$;Q3-Q4: $p=1.30x10^{-6}$

Males:
One-Way ANOVA: $p = 6,99x10^{-53}$
Post-hoc Tukey test: Q1-Q2: $p=5.58x10^{-6}$; Q1-Q3: $p=4.26x10^{-13}$; Q1-Q4: $p=4.26x10^{-13}$
Q2-Q3: $p=1.78x10^{-5}$; Q2-Q4: $p=4.26x10^{-13}$; Q3-Q4: $p=8.86x10^{-10}$

Figure 5. ROC curves constructed to determine serum fasting insulin cut-off points between healthy and sick subjects.

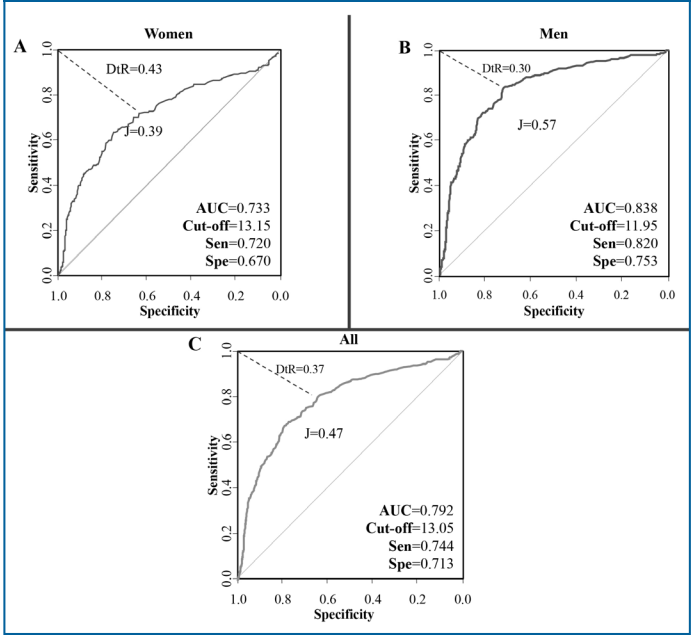


Table 6. Percentile distribution of serum fasting insulin concentration in the reference healthy population.

	Serum Fasting Insulin (uUI/mL)				
	p05th	p25th	p50th	p75th	p95th
Females	4.8	8.4	11.5	14.4	21.7
Males	4.8	6.7	9.0	12.7	21.3
Total	4.2	7.4	10.2	13.6	21.5

Table 7. Serum insulin cut-offs based on ROC Curves, Sensitivity, Specificity, Youden's Index, Positive Likelihood and Distance to the ROC Curve.

	Insulin	Sensitivity (%)	Specificity (%)	Youden Index	Distance to ROC	LR+
Women	13.05	68.9	66.3	0.35	0.45	2.04
	13.15 ¶	71.2	66.0	0.37Ψ	0.44§	2.09
	13.25	69.1	62.2	0.36	0.45	2.10
Men	11.85	82.1	72.2	0.54	0.33	2.73
	11.95¶	82.1	72.6	0.55Ψ	0.32§	2.89
	12.10	816	72.9	0.54	0.32	2.88
All	12.95	74.7	70.6	0.45	0.38	2.53
	13.05¶	**74.4**	**71.3**	**0.46Ψ**	**0.38**	**2.58**
	13.15	73.6	71.8	0.45	0.38	2.60

(¶) Selected cut-off (Basal Insulin µU/mL) based on Sensitivity, Specificity, Youden's Index and Positive Likelihood Ratios (LR+), emphasizing on highest sensitivity values.

(Ψ) Cut-off 1. asserted using the maximum Youden Index.

(§) Cut-off 2. obtained from the point closet to the ROC (0.1).

Discussion

Currently, abundant evidence supports the role of hyperinsulinemia as a risk factor for CVD[22]. Although some weight of this relationship may rely on added subsequent alterations which also predispose to CVD –such as obesity, HBP and dyslipidemia-[23]; hyperinsulinemia has been shown to display some degree of independency as a risk factor for CVD, as seen in non-diabetic subjects with this disturbance[24]. In the context of this evidence, and considering the profound deleterious metabolic and organic effects hyperinsulinemia generates are numerous[1,25], one of the main purposes of several studies worldwide is to determine appropriate cut-off values in order to detect subjects with this alteration in a timely and effective manner.

The profound genetic, metabolic, psychobiologic and behavioral influences over serum fasting insulin concentrations have been documented in several reports, which show ample variations in this parameter in diverse demographies around the world[26,27]. This great variability has hindered the agreement on a consensus on the optimal values to define hyperinsulinemia internationally[28]. Therefore, the objective of this study was to determine an appropriate cut-off for fasting serum insulin in our population through the construction of ROC Curves. This method requires the selection of adequate reference populations through specific selection criteria, in order to delimit a reference or control population constituted by "healthy" subjects, as well as a "sick" cohort; with both groups being essential to the construction of the ROC Curve and the selection of the appropriate cut-off. In our study, this selection was based in key characteristics of insulin physiology, which were extrapolated to the epidemiologic features of our population: All subjects consuming lipid or glycemic profile-altering drugs, as well as subjects with T2DM, were excluded altogether from this stage of the study, due to their overtly modified or distorted metabolic physiology. Furthermore, our "healthy" subjects were required to be non-obese, and lack history of cardiometabolic disease; whereas our "sick" subset included obese subjects with MS.

The biologic behavior of this hormone according to gender is similar to that reported in Korean and Chilean subjects[29,30], with no significant differences found between sexes. However, when stratifying the main population by age groups, fasting insulin levels appear to increase starting at 30 years in comparison with younger groups, in consonance with the results outlined by Bryhni et al.,[31]. In this regard, subjects aged 30-39 years in the main population exhibited the greatest proportions of IR and physical inactivity in all four domains of the International Physical

Activity Questionnaire, in comparison to younger individuals. Likewise, in our population the increase in fasting insulin concentration is accompanied by several other metabolic disturbances, such as progressive increases in fat mass, BMI, waist circumference, blood pressure, TAG, and LDL-C, as well as progressive decrease in HDL-C levels. Additionally, a slight lowering of fasting insulin levels is observed in subjects aged 50 and older, echoing the results by Baracco et al[32]. In our population, this may be due to greater proportions of subjects undergoing therapeutic intervention in these ages.

Obesity is a metabolic alteration intimately linked to the values of fasting insulin, fundamentally by favoring IR and the development of low-grade inflammatory states (33). Our population does not escape this extensively reported pattern[34,35], where serum concentrations of fasting insulin display an increase across BMI categories; accompanied by increasing values of HOMA2-IR and serum hs-CRP. The analysis between genders revealed Normal Weight women to have greater insulin levels than men in the same category. This finding may be due to the high prevalence of elevated waist circumference values in within this subgroup of females in comparison to their male counterpart, which also reflects in their corresponding HOMA2-IR values. On the other hand, fasting insulin levels display an inverse behavior in subjects within the Obesity Type III group, where the men's concentrations were significantly higher than the women's. In this case, age seems to play a preponderant role, with these males being significantly older than their female homologues. In addition, these men also showed an elevated prevalence of HBP, IR and hs-CRP, supporting the proposal of hyperinsulinemia as a key clustering factor for the aggregation of the components of MS, as well as low-grade inflammation[36].

Similarly, values of fasting insulinemia progressively ascended as waist circumference values increased in both sexes, as has been reported by Siani et al., in an Italian cohort[37]. This pattern reflects the close relationship between these variables, and evidences the key role played by visceral adiposity in the development of metabolic disturbances[38]. The analysis by gender showed females to have significantly higher fasting insulin levels than men within the first quartile. This phenomenon is probably due to the greater proportion of physically inactive, diabetic and insulin-resistant women in this category. Conversely, men had significantly higher levels of fasting insulin than women within the fourth quartile, accompanied by higher levels of serum hs-CRP, as well as greater proportions of hypertensive, insulin-resistant and active smoker males in this group (data not shown). Indeed, this habit may be the intermediary link between IR, hyperinsulinemia and CVD risk through several pathophysiologic pathways, particularly by favoring the development of endothelial dysfunction[39]. Furthermore, smoking worsens the low-grade inflammation observed in subjects with elevated waist circumference values due to the increased adiposity, favoring the onset of the typical components of MS[40].

The selection of a cut-off for serum fasting insulin concentration was done through the construction of a ROC Curve, which determined a value of 13.05µU/mL as the optimal point, with 74.4% sensitivity and 71.3% specificity. For clinical purposes, we propose that the cutoff point of 13 µU/mL to define hyperinsulinemia, since no statistical difference was observed between genders using the de Long Test. This threshold is similar to the one proposed by Fernández et al.[41] in a study previously conducted in our locality, using 1703 individuals whom were poorly selected. Likewise, in comparison with reports from the United States, Spain and New Zealand, our cut-off appears to be 3.3-3.5 µU/mL higher[41-43]. This may be due to socio-demographic and nutritional features endogenous to each of these territories, and other psychobiologic habits such as exercise, being physical inactivity a severe public health problem in our demography, with 59.06% of individuals being sedentary[44]; in fact, there are genetic factors that should be further studied in future investigations in our community[45,46].

Considering these figures, the appropriate assessment of cardiometabolic risk factors becomes an especially important issue in our cohort. Hyperinsulinemia is a key variable among these factors, as it represents one of the pivotal pathophysiological components whose behavior may be interpreted in order to detect and monitor the onset and evolution of MS, T2DM, CVD and several other related pathologies[47,48]. This implementation is particularly convenient in non-diabetic populations such as that studied in this report, where hyperinsulinemia and IR rarely appear isolated from each other, and elevated fasting insulin levels may be used as a surrogate in the estimation of IR[49]. To this end, the quantification of serum fasting insulin concentration is a practical and powerful tool, with the main requirement for its adequate application being the determination of specific cut-off values for each ethnic-specific population.

In conclusion, we propose an optimal cut-off of 13µU/mL for serum fasting insulin concentration in our population. This point offers sufficient sensitivity and specificity, allowing for the appropriate assessment of hyperinsulinemia in the adult population of the Maracaibo City. The determination of cut-offs specific to each demography is particularly important when evaluating risk of pathologies such as obesity, T2DM, MS and CVD, which have become severe public health issues, and which require optimal tools and resources in order to deliver effective diagnostic and therapeutic management.

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Disclosure

The authors have are no conflicts of interest to disclose.

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