STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	*(a)* Indicate the study's design with a commonly used term in the title or the abstract

« WRIST CIRCUMFERENCE : A NEW MARKER FOR ASSESSING INSULIN RESISTANCE IN SUB-SAHARAN AFRICAN WOMEN WITH POLYCYSTIC OVARY SYNDROME »

(b) Provide in the abstract an informative and balanced summary of what was done and what was found

Background

Insulin resistance, the metabolic basis for developing future cardiovascular disease, is the main complication found in 35-80% of women with Polycysticovary syndrome (PCOS). However, there is no definite consensus regarding which marker to use for its assessment in PCOS women. The aim of the current study was to assess Wrist Circumference as an easy-to detect marker of insulin resistance in Congolese women with PCOS.

Methods

Seventy-two PCOS women and seventy-one controls were enrolled in the study (mean age 24,33 ± 5,36). Fasting biochemical parameters, Homeostasis Model assessment of insulin resistance and body composition using Bioelectrical Impedance Analysis (BIA) were evaluated. Non Dominant Wrist Circumference were measured manually, as well as Waist Circumference, Hip Circumference, Height and Weight.

Results

Non Dominant Wrist Circumference was the most correlated to HOMA-IR (r = 0,346; p=0,003) than Dominant Wrist Circumference (r = 0,315; p = 0,007), Waist Circumference (r = 0,259; p = 0,028), BMI (r = 0,285; p = 0,016), WHR (r = 0,216; p = 0,068) and WHtR (r = 0,263; p = 0,027). Logistic regression showed thatNon Dominant Wrist Circumference is the best anthropometrical marker correlated to insulin resistance using HOMA-IR as biological reference marker in PCOS women (p = 0,016).The diagnostic accuracy of Non Dominant Wrist Circumference for the presence or absence of IR, using ROC curve analysis showed that the area under the ROC curve were 0.72. A cutoff value of Non Dominant Wrist Circumference of 16,3 cm, was found as the best predictor of IR in Congolese women with PCOS.

Conclusion

Non Dominant Wrist Circumference is, to date, the best anthropometrical marker of insulin resistance, in Sub-Saharan African women with PCOS.It could be suggested as an easy-to detect marker for assessing IR in women with PCOS. Our findings open new perspectivesin the correlation between insulin resistance and bone homeostasis in PCOS women.

Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported

Polycystic ovary syndrome is one of the most common endocrine disorders affecting women in reproductive age [1].

Insulin resistance, the major complication for PCOS women, is strongly associated with an increased risk of cardiovascular diseases and diabetes, and affects 35-80% of women [2-4]. Early detection of IR could help to prevent these complications. However, there is no consensus regarding the most accurate method to predict IR in PCOS women.

In the last decades, several structural body components have been evaluated in relation to insulin resistance [5-9]. Waist circumference (WC), Body mass index (BMI), Waist-to-Hip ratio (WHR), Waist-to-Height ratio (WHtR) are some of them. However, since they are all based on body fat assessment, they cannot predict acutely IR in PCOS women. Indeed, IR in PCOS has the particularity to be independent of BMI and body fat distribution [2, 3]. Consequently, a more appropriate method that should be easy to perform is sought.

Among various markers of IR, the role of anthropometric indicators is obvious in developing countries [10]. Because of poverty and lack of suitable laboratories, biological evaluation of IR based on the measurement of insulin or other biological markers is difficult. Therefore, finding a marker that is not based on the measurement of fat, and which is anthropometric and easy to perform, has become a

challenge.

Meeting this challenge has been the basis of our research.

Objectives	3	State specific objectives, including any prespecified hypotheses

Given that most of PCOS women are insulin resistant, which is independent from fat and characterized by hyperinsulinemia [2, 3, 22], we have hypothesized that in the presence of IR in Congolese PCOS women, hyperinsulinemia might induce increased bone mass that could be detected by the measurement of WrC. Wide WrC should be associated with IR in Congolese women with PCOS. Thus, WrC could be proposed as a new marker easy-to-detect for IR assessment in PCOS women.

The aim of this study is to assess the Wrist Circumference in Congolese women with PCOS in relation to IR, using the homeostasis model assessment as a biological reference marker.

Methods		
Study design	4	Present key elements of study design early in the paper

This prospective case-control study was performed from October 2015 to December 2016

Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection

This prospective case-control study was performed from October 2015 to December 2016, in Kinshasa, the capital city of the Democratic Republic of Congo, and involved 72 PCOS women and 71 controls. Since the prevalence of PCOS in the Democratic Republic of Congo is unknown, we have taken into account global prevalence, which varies between 5-10%, to determine our sample size.

Participants	6	*(a)* Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls

Cases were women with PCOS, all African in reproductive age and from a Congolese ethnic group, without hormonal treatment for the past 2 months preceding the study. Age-matched control women came from the same ethnic group. They were in reproductive age, non hirsute, without personal or family history of hirsutism and/or endocrine disorders, and without any medical treatment.

		(c) For matched studies, give matching criteria and the number of controls per case
		Age-matched control women came from the same ethnic group.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable

PCOS was defined according to the Rotterdam 2003 consensus [1] by the presence of at least two of the following three features:

a) clinical and/or biochemical signs of hyperandrogenism;
b) oligomenorrhea and/or anovulation;
c) Polycysticovaries.

Clinical hyperandogenismwas defined by the Ferriman-Gallwey score > 8.

Age-matched control women came from the same ethnic group. They were in reproductive age, non hirsute, without personal or family history of hirsutism and/or endocrine disorders, and without any medical treatment.

Women were excluded if they:

a) refused to participate in the study;
b) were pregnant or become pregnant during the study period;
c) were in peripubertalperiod;
d) were in menopause
e) were using any contraception method, hormonal treatment or insulin sensitizers.

All women were subjected to a physical examination including evaluation of blood pressure, weight, height, wrist circumference, abdominal and hip circumference. All anthropometric measurement were taken by the same examinator to minimize error.

Blood pressure was measured after a 10 minutes rest, in the sitting position, with feet on the floor and the arm supported at heart level. Normal values were considered < 130/85 mmhg [1].

Non dominant Wrist Circumference was measured with subjects in a seated position using a tape measure positioned over Lister's tubercle of the distal radius and over the distal ulna [23]

Waist circumference was measured at the end of a normal expiration, at a level parallel to the floor, at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, in the mid axillary line, using a stretch-resistant tape, with the women relaxed, in standing position with feet close together, arms at the side and body weight evenly distributed [24].

Hip circumference was measured at a level parallel to the floor, at the largest circumference of the buttocks [24].

Calculated measures included evaluation of BMI (Body Mass Index), WHtR (Waist-to-Height) and WHR (Waist-to-hip ratio).

In addition, body composition was assessed by Bioelectrical Impedance Analysis (BIA) using a body fat analyzer (OMRON BF 511®). This device measures the impedance of each body segment to 50 kHz. It uses the electrical impedance along with the height, weight, age and gender information to generate data of body composition (body fat percentage, visceral fat level and skeletal muscle percentage). Assessment was performed in the morning after a fast of ≥ 3 hours, with the use of a light dress and stripped of all metal objects. Women were instructed not to practice vigorous exercise before the test.

BMI was classified according to the WHO criteria as [25]:

a) Normal: 18.5-24.9 kg/m^2
b) Overweight: 25.0-29.9 kg/m^2
c) Obesity:
   - Class I: 30.0-35.0 kg/m^2
   - Class II: 35.1-39.9 kg/m^2
   - Class III: >40.0 kg/m^2

WHR was classified as normal if < 0.85, and substantially increased if ≥ 0.85 [24].

WC was classified as normal if <80 cm, and substantially increased if ≥ 80 cm [24].

BIOCHEMISTRY

Biological measurement concerned fasting glucose and insulin.

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group

A fasting blood sample was collected from PCOS and control subject in the morning and was centrifuged immediately. Serum was stored at -20 °C until analysis.

Blood glucose concentration was determined on the day of the blood collection by the glucose oxidase method using a glucometer (Freestyle). Insulin was measured using Elisa method.

IR was assessed by the HOMA-IR using the formula:

HOMA = [(glucose in mmol/l) x insulin in µU/ml]/ 22,5 [26, 27]

Glucose in the formula is in mmol, and has been transformed from mg/dl by the following formula:

Glucose in mmol/l = Glucose (in mg/dl) /18

Normal value of HOMA-IR was ≤ 2,74 molxµU/L² as previously reported [3].

All women with HOMA-IR > 2,74 molxµU/L² were insulin resistant [3].

Bias	9	Describe any efforts to address potential sources of bias

All anthropometric measurement were taken by the same examinator to minimize error.

In addition, body composition was assessed by Bioelectrical Impedance Analysis (BIA) using a body fat analyzer (OMRON BF 511®). Assessment was performed in the morning after a fast of ≥ 3 hours, with the use of a light dress and stripped of all metal objects. Women were instructed not to practice vigorous exercise before the test.

Study size	10	Explain how the study size was arrived at

Since the prevalence of PCOS in the Democratic Republic of Congo is unknown, we have taken into account global prevalence, which varies between 5-10%, to determine our sample size.

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

BMI was classified according to the WHO criteria as [25]:

d) Normal: 18.5-24.9 kg/m^2
e) Overweight: 25.0-29.9 kg/m^2
f) Obesity:
   - Class I: 30.0-35.0 kg/m^2
   - Class II: 35.1-39.9 kg/m^2
   - Class III: >40.0 kg/m^2

WHR was classified as normal if < 0.85, and substantially increased if ≥

0.85 [24].

WC was classified as normal if <80 cm, and substantially increased if ≥ 80 cm [24].

Normal value of HOMA-IR was ≤ 2,74 molxµU/L² as previously reported [3].

All women with HOMA-IR > 2,74 molxµU/L² were insulin resistant

Statistical methods	12	*(a)* Describe all statistical methods, including those used to control for confounding

Qualitative data have been expressed as frequency (n=number) and proportion (%). Continuous data are expressed as mean ± standard deviation. The Kolmogorov-Smirnov was used for normality analysis for the parameters. Student t-test and Chi-Square test were used for the comparisons between groups and subgroups of continuous and categorical variables, respectively. Non parametric tests were used for variable not normally distributed. Logistic regression was performed to analyze the association between study variables. The odds ratio (OR) are presented with their 95 % confidence interval (95% CI). A *P* value < 0,05 was considered as significant.

(b) Describe any methods used to examine subgroups and interactions

Student t-test and Chi-Square test were used for the comparisons between groups and subgroups of continuous and categorical variables, respectively

(*c*) Explain how missing data were addressed

(c) If applicable, explain how matching of cases and controls was addressed

Student t-test and Chi-Square test were used for the comparisons between groups and subgroups of continuous and categorical variables, respectively. Non parametric tests were used for variable not normally distributed. Logistic regression was performed to analyze the association between study variables. The odds ratio (OR) are presented with their 95 % confidence interval (95% CI).

		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed

Table 1 and Table 2 present characteristics of our study population.

Our 72 PCOS women and 71 controls participated at all stages of the study.

		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders

The mean age was similar in both groups and subgroups. There was a significant difference for the Ferriman& Gallwey (F-G) score between PCOS women and controls as well as for insulinemia and HOMA-IR ($p < 0.0001$).

PARAMETERS	PCOS (n= 72)	CONTROLS (n=71)	*P*
Age (year)	24,4 ± 5,2	24,25 ± 5,5	0,869
Menarche age (year)	12,97 ± 1,9	12,86 ± 1,8	0,723
Ferriman-Gallwey Score	8,62 ± 6,2	2,32 ± 2,3	< 0,001
Systolic blood pressure (mmHg)	109,24 ± 17,26	100,86 ±15,27	0,003
Diastolic blood pressure (mmHg)	71,84 ± 14,11	64,96 ± 12,53	0,003
BMI	25,23 ± 5,55	23,07 ± 4,52	0,013
WC (cm)	84,28 ± 13,94	78,63 ± 11,60	0,009
WHR	0,81 ± 0,06	0,80 ± 0,05	0,101
WHtR	0,51± 0,08	0,48± 0,07	0,018

Dominant WristCircumference	15,98 ± 1,15	15,53 ± 0,87	0,009
Non Dominant WristCircumference	15,80 ± 1,18	15,33 ± 0,87	0,009
Weight	66,9 ± 16,3	60,8 ± 11,8	0,013
Body fat	35,05 ± 9,6	32,37 ± 8	0,076
Muscles	26,6 ± 4,5	27,9 ± 3	0,042
Visceral fat	4,67 ± 2	3,97 ± 1,7	0,031
Glucose (mg/dl)	86,51 ± 10,3	84,3 ± 8,04	0,155
Insulin (µU/L)	14,91 ± 15,4	6,34 ± 3,8	< 0,001
HOMA-IR (mol x µU/l²)	3,40 ± 4,03	1,33 ± 0,83	< 0,001

(b) Indicate number of participants with missing data for each variable of interest

Outcome data 15* Report numbers in each exposure category, or summary measures of exposure

PARAMETERS	**PCOS IR + (n= 28)**	**PCOS IR - (n= 44)**	***P***
Age	25,07 ± 5,67	23,98 ± 4,89	0,388
Menarche age	12,71 ± 2,03	13,14 ± 1,85	0,367
Ferriman-Gallwey Score	8,04 ± 6,91	9,00 ± 5,87	0,528
Systolic blood pressure (mmHg)	114,08 ± 22,20	106,59 ± 13,43	0,087
Diastolic blood pressure (mmHg)	74,62 ± 17,42	70,32 ± 11,89	0,232
BMI	27,39 ± 6,27	23,90 ± 4,66	0,016
WC (cm)	90,09 ± 15,76	80,58 ± 11,36	0,008
WHR	0,84 ± 0,07	0,80 ± 0,06	0,014
WHtR	0,54± 0,09	0,49 ± 0,07	0,014
Dominant WristCircumference	16,49 ± 1,27	15,66 ± 0,95	0,005

Non Dominant WristCircumference	16,33 ± 1,20	15,45 ± 1,03	0,002
Weight (kg)	72,88 ± 18,56	63,26 ± 13,86	0,025
Body fat	38,50 ± 9,44	32,93 ± 9,22	0,017
Muscles	26,30 ± 3,42	26,90 ± 5,09	0,591
Visceral fat	5,48 ± 2,15	4,16 ± 1,75	0,010
Glucose (mg/dl)	93,04 ± 10,34	82,36 ± 7,99	< 0,001
Insulin (µU/L)	26,22 ± 19,99	7,71 ± 2,99	< 0,001
HOMA-IR (mol x µU/l²)	6,26 ± 5,32	1,57 ± 0,63	< 0,001

Main results	16	*(a)* Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

On the other hand, we found the presence of a strong correlation between Dominant WrC, Non-Dominant WrC, WC, BMI, Weight, WHtR and WHR. Because of the severe collinearity and high correlation, we could not use these parameters in the same regression model.

(b) Report category boundaries when continuous variables were categorized

We performed logistic regression using binary HOMA-IR (IR+: HOMA-IR > 2,74; IR-: HOMA-IR ≤ 2,74) as the dependent variable.

(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		We, then, assessed the diagnostic accuracy of Non Dominant Wrist Circumference for the presence or absence of IR, using ROC curve analysis (Figure 1).
Discussion		
Key results	18	Summarise key results with reference to study objectives
		We aimed to assess Non Dominant Wrist circumference in relation to insulin resistance in Congolese women with PCOS, using Homeostasis Model Assessment for insulin resistance as the reference biological index. We found a positive and significant correlation between Non Dominant Wrist Circumference and insulin resistance among PCOS women. Moreover, Non Dominant Wrist Circumference was the strongest marker associated with HOMA-IR, whereas Waist Circumference, BMI, WHR and WHtR were poorly associated respectively. Our observation makes the Non Dominant Wrist Circumference the best anthropometric marker of insulin resistance known to date. The novelty of the WrC as a marker of IR, is that it is based on the assessment of IR on bone, not on fat
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
		However, we recognise some limitations of our study. Among them, the limited number of our population.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
		However, we recognise some limitations of our study. Among them, the limited number of our population. We recommend large scale studies to validate our observations.
Generalisability	21	Discuss the generalisability (external validity) of the study results
		We recommend large scale studies to validate our observations.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable,

for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.