Correlations between Measurements of Body Adiposity and Metabolic Variables in Patients with Polycystic Ovarian Syndrome: A Case-Control Study

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Abstract

Objective: To study correlations between Insulin Resistance (IR) and Glucose Intolerance (GI) with body adiposity in women with Polycystic Ovary Syndrome (PCOS women) and controls.

Design, patients and Methods: A case-control study, including PCOS women (n=39) matched by Body Mass Index (BMI) and age to controls (n=35). We conducted Oral Glucose Tolerance Test (OGTT, 75g glucose), Homeostasis Model Assessment-estimated Insulin Resistance index (HOMA-IR), Electrical Bioimpedance (EB), Whole Body Densitometry (DXA), anthropometric measurements, and Visceral Adiposity Index (VAI).

Results: In PCOS women, HOMA-IR showed strong positive association with Fat Mass (FM, r=0.7164), Android Fat (AF, r=0.7729), and Fat/Lean Mass Ratio (FM/LM, r=0.7198) obtained by DXA and anthropometric measurements: Waist Circumference (WC, r=0.7423), Hip Circumference (HC, r=0.7104), BMI (r=0.7423) and Waist-To-Height Ratio (WHtR, r=0.7979). Moderate association was identified between OGTT and FM (r=0.5261), android fat (AF, r=0.5296), FM/LM (r=0.5296), WHtR (r=0.5065) and BMI (r=0.5156). In PCOS women, HOMA-IR showed moderate positive association with FM (r=0.6220) and FM/LM (r=0.6262) and negative with LM (r=-0.6220) obtained by EB. VAI presented strong (r=0.7004) and moderate (r= 0.6356) positive correlation with HOMA-IR and OGTT, respectively, in PCOS women. Subsequently, groups were subdivided into normal weight (BMI ≤ 24.9 Kg/m²) and overweight (BMI ≥ 25.0 Kg/m²). Among normal weight PCOS women, there was moderate correlation between OGTT and AF by DXA (r = 0.5308).

Conclusions: Adiposity in PCOS women could be sufficiently evaluated by BMI, WHtR, and VAI. DXA is appropriate for evaluating normal weight PCOS women due to the increased metabolic risk associated with android fat.

Keywords: Polycystic ovary syndrome, Glucose metabolism disorders, Whole body densitometry, Electrical bioimpedance, Anthropometric measurements

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in women, with an estimated prevalence between 8 and 18% [1]. Women with PCOS show higher frequency of overweight and obesity (including central obesity), and increased risk of metabolic syndrome and glucose metabolism disorders [2]. The presence of Insulin Resistance (IR) is directly related to Body Mass Index (BMI) and central obesity, that is to say, adiposity has impact on metabolic risk [3]. Patients with IR show increase in the risk of Glucose Intolerance (GI) (Relative Risk [RR] of 2.48) and type 2 diabetes mellitus (RR of 4.43) [4]. Early identification of metabolic disease is advised and periodic clinical and laboratory evaluation has been indicated to women with PCOS from their second decade of life on [5]. Appropriate methods to evaluate adiposity in PCOS women...
should present great accuracy, reproducibility, and possibility of large scale use in clinical practice, that is, easily accessible low-cost methods with fast application. Among the methods available for evaluating body composition, Whole Body Densitometry (DXA) and Electrical Bioimpedance (EB) are included. The DXA method evaluates the distribution of body fat and shows high accuracy. However, it is not portable, has elevated cost, and requires specialized operators [6]. The EB equipment is easily handled and affordable, some models are portable, in other words, they can be used at the bedside. Nevertheless, single-frequency EB equipment shows limitations in patients with alterations in body water distribution [6].

Among the most frequently used anthropometric methods for the clinical evaluation of adiposity, Body Mass Index (BMI), Waist (WC) and Hip (HC) circumference measurements, and the Waist-to-Hip Ratio (WHR) are included. However, anthropometric methods present variable reproducibility and limitations regarding the capacity of evaluating cardiometabolic risk. More specifically, individuals classified as normal weight by BMI may show increase of metabolic risk depending on body composition, that is, on the amount of Fat Mass (FM) and Lean Mass (LM) [7,8]. Moreover, it is necessary to clarify whether the indices including anthropometric variables in their formula can reproduce the results of gold standard methods of body composition evaluation. Recently, the Waist-to-Height Ratio (WHtR) and Visceral Adiposity Index (VAI) have been indicated as methods capable of evaluating abdominal fat [9,10]. Considering that in PCOS women visceral fat may elevate the risk of IR, if the association between anthropometric indices and the amount of abdominal fat is demonstrated, the indices could be used to replace DXA or EB, as a low cost alternative to evaluate metabolic risk in PCOS women.

The aim of this study was to analyze, in women with PCOS and controls, the correlations between IR and GI with body composition assessed by anthropometrics, EB, DXA, and VAI. The secondary goal was to analyze whether PCOS women show higher metabolic risk (IR, GI and VAI, anthropometric measurements, and body composition) than control women paired according to BMI and age.

Method

Observational case-control study, developed in an outpatient gynecological endocrinology unit of a tertiary university hospital in Brazil, from 2015 to 2017. The study was approved by the Ethics Committee on Research with Human Beings. All the enrolled women agreed to participate in the study and signed an informed consent. Seventy-four women were analyzed, 39 PCOS women and 35 controls, at age between 18 and 35 years old, paired according to age and BMI (maximum difference of ± 2.0 kg/m²). Each control was within the same BMI category as the PCOS case [11]. PCOS diagnosis was performed following the Rotterdam consensus criteria [12]. Women enrolled in the control group were found at the HCU-UFU outpatient family planning or general gynecology units. Control group was composed of women with spontaneous regular menstrual cycles with no history of infertility. Women using hormone medication, three months before recruitment, oral hypoglycemic drugs or weight loss inducing drugs, and pregnant or breastfeeding women were excluded from the study.

Glucose metabolism was assessed by the Homeostasis Model Assessment-estimated Insulin Resistance (HOMA-IR), calculated by the formula: HOMA-IR = glycemia (mg%) x insulin (µU/ml)/405 [13]. Women who presented HOMA-IR > 2.7 were classified as IR carriers [14]. The dosage of basal insulin was performed using the chemoluminescence method (Roche Diagnóstica©, Brazil). The oral glucose tolerance test, carried out with 75 grams of glucose, was used as GI marker. The dosages of blood glucose were performed using the hexokinase method (Roche Diagnóstica©, Brazil). The interpretation of oral glucose tolerance test results was conducted in accordance with the parameters established by the American Diabetes Association [15]. Fasting lipid profile was evaluated by the dosage of total, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) cholesterol, and by the dosage of triglycerides (enzymatic colorimetric method, Roche Diagnóstica©, Brazil). Among anthropometric variables, we gauged body weight, height, and WC measurements (performed in the midpoint between the last costal margin and the iliac crest) and HC (performed in the largest diameter of the gluteal region). For WC and HC measurements, we used an inextensible anthropometric fiberglass tape with 0.1 cm of accuracy. For the analyses of WC and HC values, we adopted the mean value of three measurements. Subsequently, WHR, HWtR and BMI values were calculated [BMI = weight (kg)/height (m)²]. BMI values were classified according to the World Health Organization [11].

The evaluation by Electrical Bioimpedance (EB) was carried out using the equipment Biodynamics 310e, TBW©, following the standardized protocol. The evaluation by the DXA method was performed with the equipment Lunar Prodigy DXA System (software version 11.20), in accordance with standardized parameters [16]. We performed measurements referring to the amount of Fat Mass (FM) and Lean Mass (LM), in grams and percentage, and FM/LM index. In addition, measurements of fat distribution in different compartments were carried out, specifically android fat (lower limit in the horizontal line that links anterior superior iliac spines, upper limit defined as 20% above the bottom edge, in parallel with the chin line and laterally by waist margins). Appendicular Fat Index (AFI) was calculated using the formula: AFI = legs FM (g) + arms FM (g) / trunk FM (g), according to equipment specifications. VAI was calculated applying the mathematical formula: VAI = [Waist/(36.58 + (1.89 x BMI))] X (Triglycerides /0.81) X (1.52/HDL cholesterol), concentrations of triglycerides and HDL cholesterol in mmol/l [17]. The results obtained for PCOS women were compared with the results obtained for control women. Next, the following subgroups were constituted according to BMI categories: PCOS normal weight (PCOS-Nw, n = 20) and control normal weight (C-Nw, n = 16), with BMI up to 24.9 kg/m²; PCOS overweight (SOP-Ow, n = 19) and control overweight (

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(C- Ow, n = 19), in which BMI was ≥ 25.0 kg/m².

Statistical Analysis

Statistical analyses were performed using the program GraphPad Prism® version 6.00 for Windows; GraphPad Software, La Jolla California USA (www.graphpad.com) and the program BioEstat 5.0 (http://www.mamiraua.org.br/pt-br/downloads/programas/). Variables were studied using D’Agostino and Pearson method in terms of distribution pattern; they were then categorized into parametric and non-parametric variables. Continuous parametric variables were compared using paired Student test and non-parametric variables were compared using paired Wilcoxon test. For the comparison between more than 2 groups of variables, ANOVA test was employed, with Tukey post-test for normal distribution variables, and Kruskall-Wallis with Dunn post-test for non-parametric variables. For correlation analyses, Pearson correlation coefficient was used when the analysis was performed between two parametric variables, and Spearman correlation coefficient, when the analysis was performed between two non-parametric variables. The correlation coefficient (r) was considered weak when the identified value was between 0.3 and 0.5; moderate when between 0.5 and 0.7; strong when between 0.7 and 0.9; and very strong when higher or equal to 0.9. Comparison among frequencies of discrete variables was carried out using Fisher test. Analyses were considered significant when p < 0.05 was identified, using two-tailed tests.

Results

PCOS and control groups showed similar mean age (25 versus 26 years, p = 0.5024). The medians of the analyzed anthropometric variables, including BMI, were similar between groups. No difference was identified for body composition evaluation by EB and DXA methods, except for the AFI, which was significantly lower in PCOS women (p = 0.0435) (Table 1). For fasting blood glucose, HOMA-IR index, serum lipids, as well as IR and GI frequency, similar mean values were demonstrated for both groups. PCOS women showed higher mean value for blood glucose after 2-hour overload (OGTT) (p = 0.0090) (Table 1).

|                | PCOS (n=39) | Control (n=35) | p    |
|----------------|-------------|----------------|------|
| **Anthropometric variables** |             |                |      |
| BMI (kg/m²)    | 24.43 (20.90 - 33.84) | 24.20 (21.75 - 31.00) | 0.728 |
| Waist (cm)     | 83.50 (72.00 - 103.30) | 81.00 (72.00 - 93.50) | 0.466 |
| Hip (cm)       | 102.00 (95.63 - 117.00) | 101.00 (95.00 - 110.00) | 0.9966 |
| WHR            | 0.8109 (0.7553 - 0.8715) | 0.8000 (0.7577 - 0.844) | 0.2067 |
| WHtR           | 0.5138 (0.4441 - 0.6284) | 0.5000 (0.4565 - 0.5994) | 0.2776 |
| **Bioimpedance** |             |                |      |
| FM (%)         | 30.80 (26.00 - 39.30) | 31.40 (25.60 - 36.40) | 0.6829 |
| LM (%)         | 69.20 (60.70 - 74.00) | 68.60 (63.60 - 74.40) | 0.6829 |
| FM/LM          | 0.4450 (0.3500 - 0.6425) | 0.4600 (0.3400 - 0.5700) | 0.5909 |
| **Densitometry** |             |                |      |
| FM (%)         | 39.20 (33.30 - 48.70) | 43.40 (33.30 - 49.00) | 0.4413 |
| FM/LM          | 0.6400 (0.5000 - 0.9500) | 0.7100 (0.5000 - 0.9100) | 0.7273 |
| Android Fat (%) | 46.70 (34.40 - 54.90) | 46.20 (35.90 - 53.90) | 0.7425 |
| Appendicular Fat Index | 0.9200 (0.7400 - 1.1100) | 1.040 (0.9500 - 1.1200) | **0.0435** |
**Table 1**: Comparative analyses between women included in PCOS and Control groups in relation to anthropometric variables, body composition by DXA and EB methods.

In FM evaluation, FM/LM ratio, and android fat, by EB and DXA methods, higher values were identified in the overweight subgroups, with or without PCOS. The AFI was lower in women included in the PCOS-Ow group, in relation to all groups: PCOS-Nw (p = 0.0002), C-Nw (p < 0.0001) and C-Ow (p = 0.0134). No differences were identified for the values of AFI among the control women included in C-Nw or C-Ow groups, neither in the values of other measurements performed to evaluate body composition, by EB and DXA methods, in all groups (Table 2).
Table 2: Comparative analyses performed between women included in PCOS and Control groups, subdivided in normal weight and overweight in relation to anthropometric variables, body composition by DXA and EB.

| Variable                          | Control Normal Weight | Control Overweight | PCOS Normal Weight | PCOS Overweight |
|-----------------------------------|-----------------------|--------------------|-------------------|-----------------|
| **BMI (kg/m²)**                   |                       |                    |                   |                 |
|                                   | 21.16 (19.52 - 23.15) | 33.84 (30.20 - 37.63) | 22.14 (20.55 - 23.03) | 31.03 (27.55 - 37.88) |
| **WHtR**                          | 0.4509 (0.4273 - 0.4906) | 0.622 (0.5849 - 0.6810) | 0.4575 (0.4348 - 0.4865) | 0.6066 (0.5378 - 0.6552) |
| **Bioimpedance**                  |                       |                    |                   |                 |
| **FM (%)**                        | 27.05 (25.48 - 30.10) | 39 (33.63 - 41.55) | 25.8 (23.60 - 29.70) | 37.5 (34.00 - 41.18) |
| **LM (%)**                        | 72.95 (69.90 - 74.53) | 61 (58.45 - 66.38) | 74.2 (70.30 - 76.40) | 62.5 (58.83 - 66.00) |
| **FM/LM**                         | 0.37 (0.34 - 0.43) | 0.635 (0.50 - 0.71) | 0.35 (0.31 - 0.42) | 0.6 (0.51 - 0.70) |
| **Densitometry**                  |                       |                    |                   |                 |
| **FM**                            | 33.8 (28.38 - 37.33) | 48.7 (45.70 - 52.70) | 34.8 (29.70 - 41.10) | 48.4 (45.78 - 54.53) |
| **Android Fat**                   | 34.75 (29.20 - 41.43) | 54.9 (52.60 - 59.60) | 36 (30.90 - 43.90) | 53.9 (51.80 - 57.05) |
| **FM/LM**                         | 0.51 (0.3925 - 0.5925) | 0.95 (0.8400 - 1.1100) | 0.53 (0.4200 - 0.6700) | 0.935 (0.8425 - 1.198) |
| **Appendicular Fat Index**         | 1.015 (0.9000 - 1.205) | 0.81 (0.6500 - 0.9300) | 1.07 (1.010 - 1.150) | 1 (0.8550 - 1.100) |

DXA: Whole Body Densitometry; EB: Electrical Bioimpedance; C-Nw: Controls Normal Weight; C- Ow: Controls Overweight; PCOS-Nw: PCOS Normal weight; PCOS- Ow: PCOS Overweight; WHR: Waist-to-Hip Ratio; BMI: Body Mass Index (weight/height²); WHtR: Waist-to-Height Ratio; FM: Fat Mass; LM: Lean Mass; FM/LM: Fat Mass/Lean Mass; Appendicular Fat Index: arms FM + legs FM/trunk FM; *p < 0.05.

Results were expressed in median (p25 - p75).
Among women with PCOS, strong correlation between HOMA-IR and anthropometric measurements WC, HC, BMI, WHR was demonstrated. There was a strong correlation between HOMA-IR and body composition evaluation by DXA, in relation to FM/LM index and measurements of FM and android fat. Moderate correlation between HOMA-IR and FM/LM index and measurements of FM and LM was identified in body composition evaluation by EB (Table 3).

### Table 3: Correlation analyses in PCOS and Control groups between HOMA-IR and OGTT with anthropometric variables and body composition by DXA and EB.

Among PCOS women, moderate correlation was demonstrated between OGTT and the anthropometric variables BMI and WHtR, and the variables FM, FM/LM, android fat and appendicular fat index obtained through DXA (Table 3). Specifically, for women classified as PCOS-Ow, there was moderate correlation between HOMA-IR and WHtR ($r = 0.6593$), while for PCOS-Nw, moderate correlation between OGTT and android fat was observed through DXA ($r = 0.5308$) (Figure 1).
Weak correlation between OGTT and FM/LM index and FM and LM measurements was identified in body composition evaluation by EB (Table 3). We demonstrated that VAI showed strong correlation with HOMA-IR and moderate correlation with OGTT (Figure 2) among women with PCOS.

Figure 1: Correlation analyses between HOMA-IR and Waist-to-Height Ratio (WHtR) variables in overweight subgroups and between Oral Glucose Tolerance Test (OGTT) with android fat in normal weight subgroups between women included in PCOS and Control groups.

Figure 2: Correlation analyses between HOMA-IR and Oral Glucose Tolerance Test (OGTT) with Visceral Adiposity Index (VAI) between women included in PCOS and Control groups.
Discussion

In the present study, it was demonstrated that for women with PCOS HOMA-IR showed strong correlation with some parameters of body composition evaluation by DXA (FM/LM index and FM and android mass measurements), with most anthropometric measurements (WC, HC, BMI, WHtR) and with VAI. In accordance with the results demonstrated in our study, Carmina, et al. [18] also identified in PCOS women strong correlation between lower insulin sensitivity and central abdominal fat measurements by DXA. Despite the high cost of the method, DXA has been recommended in clinical studies on body composition evaluation due to its elevated accuracy [17,18]. In contrast, the use of anthropometric methods for evaluating IR risk in PCOS women is a subject of criticism in relation to the accuracy of different anthropometric variables [19-21]. The American Association of Clinical Endocrinologists proposes the use of WHR to evaluate IR risk in PCOS women [22]. However, in this study, we demonstrated that WHR was not the most sensitive marker for the evaluation of metabolic risk, similar to previously reported results by Lord, et al. [20]. More recently, as demonstrated in our study, WHtR has been pointed out as a better marker of metabolic risk in women with PCOS and in controls [9,23].

In body composition evaluation thorough single-frequency EB, it was demonstrated that HOMA-IR shows moderate correlation with FM/LM index and with FM and LM measurements, that is, less evident correlations than those demonstrated for DXA and for anthropometric methods. In disagreement with the results presented in this study, Ezeh, et al. [24] proposed that EB could be used advantageously in substitution of DXA, without sensitivity loss in body composition evaluation and with lower financial impact. In our study, we demonstrated that VAI showed strong correlation with HOMA-IR and moderate correlation with OGTT in women with PCOS. These results are concordant with the results obtained by Oh, Sung and Lee [10] who reported strong correlation between VAI and findings using tomography, considered a gold standard method for evaluating visceral fat and predicting IR. VAI has also been described as indicator of visceral fat effects, showing correlation with cardiometabolic risk in the general population [23].

The present study demonstrates that in women with PCOS 2-hour blood glucose after overload showed moderate correlation with all the parameters of body composition evaluation by DXA (FM and android fat measurements and FM/LM and appendicular fat indices), with anthropometric measurements (BMI and WHtR), and with VAI. In addition, it demonstrated that 2-hour blood glucose presented weak correlation with the parameters of body composition evaluation by EB (FM/LM Index). Although other investigators have also performed OGTT and investigated parameters of body composition evaluation by DXA in women with PCOS [25], no study specifically analyzing the correlations between anthropometric measurements and DXA parameters with 2-hour glucose in PCOS women has been found. Bi, et al. [26] investigated the correlations between body composition evaluation performed by DXA and anthropometric measurements with OGTT results in obese women not diagnosed with PCOS. The investigators concluded that DXA shows results superior to anthropometrics in the correlation with glucose metabolism disorders in obese women. In disagreement with the results obtained in our study, Ezeh, et al. [24] identified strong correlation between the parameters of body composition evaluation obtained using EB and intravenous glucose infusion test in PCOS women.

It was also demonstrated, in this study, that women included in the overweight PCOS group showed lower appendicular fat index than the women included in the other groups, more specifically, overweight controls, normal weight PCOS, and normal weight controls. To the best of our knowledge, it represents an unprecedented result in the literature, although greater trunk and lower limbs FM ratio has been demonstrated in PCOS [27]. Appendicular fat has been considered a protective factor against metabolic syndrome in the general population [28], once the predominance of fat mass in the appendices presumes lower amount of visceral fat, in other words, lower cardiovascular risk. The demonstration of lower AFI values in women with PCOS suggests that AFI is a marker of relevant cardiometabolic risk, peculiar to this condition. In the study, we demonstrated that PCOS women showed greater mean value for 2-hour blood glucose after overload (OGTT), what is in agreement with the results in the literature [25,29]. Furthermore, WC and WHR mean values were similar between PCOS women and controls, what goes against the results from other investigators [24,30]. In the attempt to clarifying these results, we may claim that the women enrolled in the sample of the present study were fairly young. PCOS phenotypic manifestations might take more time to appear, once they are influenced by years of chronic exposure to elevated levels of androgen [31]. The demonstration that WC and WHC were similar between PCOS and control women, concomitantly with the identification of lower AFI in PCOS-Ow women, suggests that phenotypic manifestations are more subtle in young patients, and then DXA is more sensitive to this evaluation. The rigor in pairing the groups of the study, respecting the range of 2 kg/m² in BMI, as well as the observance of the same BMI category at recruitment, is another possible explanation. An example of how the control for confusing variables might interfere in the outcomes is the study by Pazderska, et al. [32]. These investigators identified that fasting insulin, triglycerides, and HOMA-IR were higher in PCOS women than in controls paired according to BMI. However, all the differences they found did not persist after performing the control to WC. In our study, the strict pairing resulted in WC mean values similar among groups. It might have an explanation for the similarity among the other metabolic parameters between PCOS and controls, including for IR.

IR prevalence was similar among PCOS and control women, while OGTT was higher in PCOS women. Probably, when there is IR, the presence of PCOS accelerates the evolution of metabolic disturbance, facilitating the progression towards GI in women with
The same BMI. HOMA-IR elevated with the increase of BMI both in PCOS and in control women, a fact that is in agreement with the results from other researchers [33]. It was evaluated whether EB and DXA could add relevant information to the care of normal weight women with PCOS. It is reported in the literature that in the normal weight PCOS group, when there is predominance of visceral fat, there is lower sensitivity to insulin [25,34]. Therefore, phenotypic heterogeneity in normal weight PCOS could have direct impact on the risk of glucose metabolism disorders, particularly bringing benefits to these patients thorough the methods of body composition evaluation [18]. In accordance with the hypothesis formulated, our study demonstrated moderate correlation between android fat and GI only in normal weight PCOS women, but not in control women at the same weight range. The major strengths of this study were the rigorous control in group pairing and the use of high accuracy methodology (DXA). In addition, the study has an eminently practical character, aiming to offer answers in terms of the benefit of adopting methods of body composition evaluation in clinical care routine of PCOS patients. As main fragility, we must mention sampling size, what has limited the analysis of subgroups per BMI range.

Conclusion

Adiposity evaluation of PCOS women in the clinical setting can be carried out based on VAI calculation and anthropometric measurements, among them BMI and WHtR stands out given their correlation with metabolic risk. The best practical application of body composition methods lies on the evaluation of normal weight PCOS women, once in the presence of larger amount of android fat by DXA the cardiometabolic risk increases indeed. Single-frequency EB shows limited contribution in adiposity evaluation in PCOS women.

Statement of Authorship

Camila Toffoli Ribeiro, Catarina Mendes Silva and Nayara Bernardes da Cunha participated in the study design, statistical analyses, interpretation of data, and manuscript drafting. Daurea Abadia De-Souza, Rosana Maria dos Reis and Ana Carolina Japur de Sá Rosa e Silva participated in the study design, data interpretation and critical revision of the manuscript for important intellectual content. All authors approved the final version for submission.

Conflict of Interest Statement and Funding Sources

None of the authors had any personal or financial conflict of interest.

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