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Research Article

Nitric Oxide Metabolites (Nitrite and Nitrate) In Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders affecting women of reproductive age, is associated with comorbidities (metabolic syndrome, dyslipidemia and diabetes) that may contribute to increased risk of cardiovascular disease (CVD), which is one of the leading causes of female morbidity and mortality. A significant association between nitric oxide metabolites (NOx) and inflammatory conditions is observed in literature. The present study was designed to determine the concentrations of NOx in two different groups of PCOS women, with or without insulin resistance (IR) based on the Homeostasis Model Assessment (HOMA). We developed a cross-sectional study enrolling 56 women: 26 PCOS with IR and 30 controls (PCOS without IR). All of them underwent anamnesis, physical examination, transvaginal ultrasound and blood samples. NOx concentrations were significantly higher in PCOS women with IR compared to the group without IR (37.1 ± 13.4 and 28.5 ± 7.4, respectively. P=0,06). This datum might suggest, as far as NOx is concerned, a higher CVD risk among PCOS women with IR. However, more studies including a larger sample of women are required to confirm these findings.

Keywords: Polycystic Ovary Syndrome; Insulin Resistance; Body Mass Index; Cardiovascular Disease; Nitric Oxide Metabolites

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, and is frequently but not consistently associated with insulin resistance (IR) and compensatory hyperinsulinism (HI) [1]. Insulin resistance (IR) is a well established feature among many patients with PCOS, affecting 44% to 70% of them, with an esti-

mated of 5- to 8-fold increased risk of type 2 diabetes mellitus (T2DM) compared with age- and weight-matched controls [2]. This wide range may be due to several factors, including the heterogeneity of the diagnostic criteria for PCOS employed in these studies, the genetic background among the assessed population and differences in the methods used for defining IR [3]. More than 50% of women with PCOS are insulin resistant, with an estimated that they have a 5- to 8-fold increased risk of type 2 diabetes mellitus (T2DM) compared with age- and weight-matched controls [4].

Nitric Oxide (NO) is produced through the oxidation of L-arginine in different cells by a family of enzymes, the nitric oxide synthases (NOS), subdivided in three major classes: neuronal-NOS (nNOS,type 1), inducible-NOS (iNOS, type II) and endothelial-NOS (eNOS, type III) [5]. Production of NO in the vasculature is generally considered to be a "good" thing, leading to vasodilation, attenuated release of endothelin-1 and thromboxane A2, inhibition of platelet aggregation, and inhibition of smooth muscle proliferation, and stimulation of endothelial cell proliferation/angiogenesis [6]. In several clinical conditions the inducible NOS (iNOS) is hyperactivated and this event is especially associated with the increase of cytokines such as TNFα, IL-1β and interferon [7,8,9]. The activation of iNOS generates NO in the measure of up 1000-fold greater than nNOS or eNOS . This accelerated NO synthesis has however an opposite effect because NO interacts with the superoxide anion (O2−) to produce peroxynitrite and other oxidants involved in tissue injury [10]. NO metabolites (NOx), such as nitrite (NO2−) and nitrate (NO3−), usually evaluated together and expressed as NOx may play a positive role in several clinical conditions. Some authors observed a significant positive association between NOx and inflammatory conditions (arterial hypertension, chronic kidney disease, metabolic syndrome, systemic sclerosis and in dialyzed and acute myocardial infarction subjects), which are associated with increased atherogenic risk [11].

Over the past decade, several serious metabolic complications have been associated with PCOS, and the potential long-term metabolic and vascular consequences of PCOS continue to attract considerable interest. Considering this information, we examined the behavior of NOx in two different groups of PCOS women, regarding the existence or not of the insulin resistance.

Methods

This study was conducted at the Department of Obstetrics and Gynecology, Santa Casa São Paulo-Faculty of Medical Science, São Paulo-Brazil. The Institutional Review Board approval was obtained from the Ethical Committee of University. Twenty six women with PCOS and IR, and 30 PCOS without IR volunteered to participate in the study. An informed consent was obtained from each patient. For this study, we excluded women who were taking hormonal contraceptives or medications that would affect carbohydrate metabolism or immune function for at least 3 months before study participation. Others exclusion criteria were: smoking, hypertension, diabetes, inflammatory disease, dyslipidemia and thyroid´s diseases. PCOS were diagnosed on the basis of the Rotterdam consensus [12]. The IR was diagnosed using the Homeostasis Model Assessment (HOMA) and taking as reference a value equal or greater to 3 [13]. HOMA index was calculated by multiplying insulin (μU/ml) by glucose (mg/dL) and dividing this product by 405 [14].

Subjects were interviewed privately, using pretested questionnaires. Anthropometric measurements included body weight, height, body mass index (BMI) and waist circumference (WC). Weight was measured, while subjects were minimally clothed without shoes, using digital scales and recorded to the nearest 100g. Height was measured in a standing position, without shoes, using a tape meter while the shoulders were in a normal alignment. BMI was calculated as weight (kg) divided by square of height (m^2). WC was measured at the midpoint between the lower rib margin and the iliac crest and was recorded to the nearest 0,1cm. To measure blood pressure, subjects rested for 15 minutes, blood pressure was taken in a seated position using a standard mercury sphygmomanometer. Hirsutism was defined as a modified Ferriman-Gallwey score 8 or more [15]. Blood samples were taken after 12 overnight fasting and until the ninth day after the onset of menses. Total cholesterol, HDL-cholesterol, triglycerides and glucose were determined by colorimetric-enzymatic methods using the Roche Diagnostics (Mannheim, Germany) COBAS INTEGRA 400 PLUS. LDL-cholesterol was estimated indirectly using: LDL= total cholesterol – HDL – triglycerides/5. Total testosterone (TT), sex hormone-binding globulin (SHBG), insulin and dehydroepiandrosterone sulfate (DHEAS) were determined by electrochemiluminescence immunoassay using the Roche Diagnostics (Mannheim, Germany) ELECSYS 2010. Free testosterone (FT) was estimated using TT and SHBG [16]. Androstenedione (Δ_4) was measured by chemiluminescent enzyme immunoassay using the IMMULITE 2000 from Siemens Healthcare Diagnostics Products (United Kingdom). Dehydroepiandrosterone (DHEA) and 17OH progesterone were measured by enzymatic immunoassay using the ACTIVE from Diagnosis System Laboratories (Texas, USA). NOx was determined using a total nitric oxide assay kit from Assay Designs (Ann Arbor, MI-USA). NO Metabolites. Considering that in vivo NO has avery short life (less than 0.1 sec) and it is converted intonitrite (NO2−), which has a half-life of few minutes, and into the more stable nitrate (NO3−), NOχrepresents almost only the nitrate concentration. In the laboratory method adopted by us at first nitrate was converted into nitrite by a nitrate reductase, and then nitrite was assessed by spectrophotometry after the addition of the Griess reagent that absorbs visible light at 540nm [17].

Statistical Analysis

The values were expressed as mean ± SD. Student t-test and covariance (ANCOVA) were used for comparing biomarkers

between PCOS women with and without IR in un-adjusted and BMI adjusted models respectively. The correlation between variables was tested using a Person correlation test, "r^2"= value of the correlation coefficient, and "p"= descriptive level. All analyses were performed using the Statistical Package for the Social Sciences (SPSS version 15.0, Chicago, USA). Data were considered to be significant at two-tailed P-value<0.05.

Results

Both groups were similar in age. Examining the anthropometric profile and hirsutism of PCOS women subdivided according to the presence or not of IR, we observed that waist circumference, BMI and the Ferriman-Gallwey score were significantly higher in PCOS with IR (Table I). Considering the glycometabolic pattern, PCOS with IR had a higher and significantly concentration of insulin and triglycerides. Androstenedione (Δ_4), 17OH Progesterone, Free testosterone and cortisol were also higher and significantly in PCOS with IR (Table II). NOx concentrations were significantly higher in PCOS women with IR. After BMI and WC adjustment serum NOx values remained higher, but not statistical significant (table III). Examining the linear regression among NOx and all the parameters previously considered, in all women with PCOS (combined groups) we found a positive correlation between NOx and IBM (r = 0,349 ; P =0,008). Subdividing PCOS subjects according to the presence or not of IR, we observed a positive correlation between NOx and IR (r = 0,493; P =0,006).

Discussion

PCOS is associated with comorbidities (metabolic syndrome, dyslipidemia and diabetes) that may contribute to increased risk of cardiovascular disease (CVD), which is one of the leading causes of female morbidity and mortality [18]. Given the high prevalence of PCOS, this condition may potentially account for a significant proportion of atherosclerotic heart disease observed in women. Several studies have examined the prevalence of markers of subclinical CVD in women with PCOS. There is evidence for impaired endothelial function, an early marker of atherosclerosis, in young women with PCOS [19]. Elevated insulin and glucose concentrations are associated with increased CVD risk, regardless of diabetes. Indeed, evidence accumulated over the last decade has shown that loss of insulin signaling in the endothelium accelerates atherosclerotic lesions and vascular dysfunction [20]. Insulin resistance is associated with endothelial dysfunction and loss of NO biological activity or biosynthesis is a central mechanism of endothelial dysfunction. Despite the protective role of NO in cardiovascular function, evidence indicates that higher levels of NOx are present in subjects with metabolic syndrome and diabetes, disorders that are both associated with increased atherogenic risk [21]. PCOS is a proinflammatory state as evidenced by elevated plasma concentrations of a number of inflammatory mediators of atherogenesis [22]. Due to very short half-life, determination of the NO itself is difficult; consequently, measurement of the circulatory stable end products of NO, nitrite

TABLE I	PCOS	Mean	SD	Median	Minimum	Maximum	P
Age (years)	without IR	29.3	5.6	28.5	20.0	41.0	0,272
	with IR	27.6	5.4	29.0	16.0	36.0	
WC (cm)	without IR	83.8	9.6	29.0	62.0	101.0	**<0,001**
	with IR	97.3	10.1	82.0	75.0	112.0	
BMI (Kg/m2)	without IR	23.8	2.9	98.0	17.1	28.2	**<0,001**
	with IR	28.9	4.02	90.5	21.2	35.0	
FERRIMAN-GALLWEY	without IR	9,333	6,008	9,00	0,00	24,00	**0,039**
	with IR	12,885	6,556	12,00	3,00	25,00	

Anthropometric profile and Hirsutism (FERRIMAN-GALLWEY≥8). WC: waist circunference. BMI: body mass index.

TABLE II	PCOS	Mean	SD	Median	Minimum	Maximum	P
S-DHEA (µg/dL)	without IR	178,540	118,865	149,50	28,60	552,00	0,485
	with IR	200,308	111,302	186,50	20,70	378,20	
DHEA (ng/mL)	without IR	17,150	14,626	11,00	4,50	80,00	0,071
	with IR	24,885	16,820	23,75	4,50	62,50	
Δ4 (ng/mL)	without IR	2,233	1,209	2,13	0,30	5,69	**0,007**
	with IR	3,612	2,334	3,41	1,05	13,54	
17OH Progesterone (ng/mL)	without IR	0,545	0,347	0,46	0,18	1,90	**0,025**
	with IR	0,766	0,369	0,66	0,24	1,40	

TT (ng/mL)	without IR	0,296	0,239	0,25	0,02	0,87	0,154
	with IR	0,399	0,292	0,36	0,06	1,31	
FT (pmol/L)	without IR	15,367	15,294	12,50	0,00	70,00	**0,018**
	with IR	28,346	24,151	22,50	1,00	116,00	
SHBG (nmol/L)	without IR	51,913	32,534	46,15	13,80	157,20	0,111
	with IR	36,942	36,702	29,50	8,00	200,00	
INSULIN (µU/mL)	without IR	7,130	2,596	7,00	1,50	12,20	**<0,001**
	with IR	28,285	23,530	20,30	13,70	108,30	
HDL cholesterol (mg/dL)	without IR	49,467	11,045	48,00	32,00	88,00	0,636
	with IR	47,846	14,385	45,00	25,00	98,00	
LDL cholesterol (mg/dL)	without IR	86,833	24,169	89,00	42,00	127,00	0,381
	with IR	93,885	35,262	90,00	25,00	179,00	
TG (mmol/L)	without IR	0,706	0,243	0,68	0,28	1,55	**0,006**
	with IR	0,960	0,390	0,94	0,47	1,86	
Cortisol (µg/dL)	without IR	10,283	3,258	10,40	5,20	18,20	**0,050**
	with IR	12,388	4,567	11,75	5,70	24,30	
FG (mg/dL)	without IR	87,667	6,733	87,50	75,00	104,00	0,167
	with IR	90,462	8,204	91,00	63,00	105,00	

Blood samples. S-DHEA: dehydroepiandrosterone sulfate. DHEA: dehydroepiandrosterone. Δ4: Andostenedione. TT: total testosterone. FT: free testosterone. SHBG: sex hormone binding globulin. HDL: high density lipoprotein. LDL: low density lipoprotein. TG: triglycerides. FG: fasting glucose.

TABLE III	PCOS	Mean	SD	Median	Minimum	Maximum	P
Nox (nmol/mL)	without IR	28,502	7,467	26,54	18,84	50,87	**0,006**
	with IR	37,173	13,429	35,68	19,22	92,26	

NOx: Nitric oxide metabolites (nitrite and nitrate)

P= 0,169 (not significant) after BMI adjustment

P= 0,242 (not significant) after CW adjustment

and nitrate (NOx), are most often used to evaluate NO production.

Taking into consideration the above information, we examined 26 PCOS women with IR compared to 30 PCOS without IR (control group). NOx were chose as a precocious inflammatory marker and evaluated in both groups. In this study, serum NOx levels were significantly higher in PCOS with IR. Our results also showed a positive correlation between NOx and BMI and IR. These data agree with the observations of others who studied NOx in many proinflamatory conditions. To our knowledge, this is the first study to investigate the NOx among PCOS women. In our study, although there were no significant differences in NOx after adjustment for BMI and WC. This might suggest that obesity and abdominal adiposity could play a more important role, as far as CVD is regarded, rather than the PCOS *"per se"*. However, more studies which correlates PCOS and NOx, including a larger sample of women, are required to confirm these findings.

In conclusion, the subdivision of PCOS women according to the HOMA-IR allows the discrimination of 2 subgroups and the demonstration that in those with IR the NOx levels are significantly higher than in the subgroup without IR.

Declaration of Interest

The authors report no declarations of interest.

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