Universidade Federal do Rio Grande do Sul

Faculdade de Medicina

Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

Atividade autonômica, medida pela variabilidade da frequência cardíaca em pacientes com diferentes fenótipos da Síndrome dos Ovários Policísticos

Kristhiane Di Domenico Cunha

Porto Alegre, maio de 2012

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, como requisito parcial para obtenção do título de Mestre.

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Coorientador: Prof. Dr. Ruy Silveira Moraes Filho

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Esta dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma revisão geral e um manuscrito sobre o tema da dissertação:

- Revisão: Risco metabólico e cardiovascular em mulheres com a Síndrome dos Ovários Policísticos
- Artigo original: Cardiac autonomic modulation in polycystic ovary syndrome: does the phenotype matter?

SUMÁRIO

Risco metabólico e cardiovascular em mulheres com a Síndrome dos Ovários Policísticos

A Síndrome dos Ovários Policísticos (PCOS) é a endocrinopatia com maior prevalência em mulheres na idade reprodutiva cujas principais características clínicas são anovulação crônica e manifestações de hiperandrogenismo (1, 2, 3). Atualmente ela é definida pela presença de pelo menos dois dos seguintes critérios: a) oligo ou anovulação; b) hiperandrogenismo clínico e/ou bioquímico; c) presença de ovários policísticos no ultrasom (12 ou mais folículos com 2 a 9 mm ou aumento do volume ovariano (> 10 cm^3) em pelo menos um ovário), conforme os critérios de Rotterdam (2). Durante a avaliação diagnóstica, devem ser excluídas outras patologias que apresentem características semelhantes como tumores secretores de androgênios, síndrome de Cushing, hiperplasia adrenal congênita, hiperprolactinemia e alterações tireoidianas.

A utilização desses critérios determinou o reconhecimento de novos fenótipos da PCOS, além dos descritos pelos critérios prévios do National Institutes of Health (NIH), de 1990 (4). Estes novos fenótipos incluem, por exemplo, mulheres hiperandrogênicas com ciclos menstruais ovulatórios ou mulheres com ciclos anovulatórios sem a presença de hiperandrogenismo. Estes grupos usualmente apresentam-se com formas mais brandas da síndrome e podem diferir da forma "clássica" de PCOS (pacientes com ciclos anovulatórios e hiperandrogênicas) nos níveis de gonadotrofinas, secreção de hormônios esteróides, severidade da resistência insulínica, assim como no aumento do risco cardiovascular (5, 6, 7).

O hiperandrogenismo, frequentemente presente nas pacientes com PCOS, está associado às alterações clínicas e metabólicas, em especial à resistência insulínica (RI), encontradas neste grupo especial de mulheres, principalmente na forma "clássica" da doença (8, 9, 10). Estudos sugerem que a resistência insulínica e a consequente hiperinsulinemia compensatória possam desempenhar um papel central na etiopatogenia do PCOS (11,12,14, 15), apesar de ainda estar indefinido o mecanismo preciso desta associação (16,17). As pacientes com PCOS apresentam RI independente da obesidade, sendo que a presença concomitante das duas características leva a uma piora na sensibilidade insulínica, elevando ainda mais o risco para

Diabetes Mellitus tipo 2 (DM2) e possivelmente de doenças cardiovasculares (DCV) nestas pacientes (18, 19, 20).

Aproximadamente 60% das mulheres com PCOS apresentam sobrepeso ou obesidade, na sua grande maioria com padrão de distribuição central (22). O hiperandrogenismo, um dos mais importantes achados da PCOS, está associado a um acúmulo preferencial de gordura nas porções superiores do corpo, independente do IMC (Índice de Massa Corporal), chamada de distribuição de gordura andróide (3). Este tipo de obesidade está associado à hiperinsulinemia, tolerância diminuída à glicose (IGT), DM2, aumento de produção de hormônios androgênicos, diminuição de Sex hormone-binding globulin (SHBG), com consequente elevação de níveis de testosterona e estradiol livres (23). Além disso, é bem estabelecido que a obesidade está associada à maior incidência de anovulação, aborto espontâneo e complicações gestacionais tardias (pré-eclâmpsia e diabetes gestacional). Tem sido descrito que mulheres obesas com PCOS, após diminuição de 5% do peso corporal apresentam uma maior taxa tanto de ovulação quanto de gestação espontânea. (24).

Estudos avaliando o papel da obesidade na PCOS nos diferentes fenótipos da síndrome demonstram que os valores de IMC parecem diferir significativamente entre os subgrupos, com valores maiores em mulheres com a forma "clássica" em comparação às que não apresentam hiperandrogenismo (6, 25). Além disso, outros parâmetros metabólicos, como alterações do perfil lipídico, e resistência insulínica, assim como marcadores inflamatórios, como proteína C reativa (PCR) e contagem de leucócitos totais, permanecem presentes nas pacientes com PCOS clássico quando comparadas às pacientes de fenótipos mais brandos da síndrome, mesmo após ajuste para IMC (6, 40), sugerindo que as diferenças encontradas entre os fenótipos de PCOS não se explicam puramente pelo aumento da gordura corporal.

A PCOS tem sido fortemente associada a uma alta prevalência de dislipidemia, podendo estar presente em até a 70% das pacientes, com aumento de LDL-c e triglicerídeos, e diminuição de HDL-c. (31). Especula-se que a dislipidemia presente neste grupo de mulheres apresenta-se com perfil ainda mais aterogênico do que em mulheres sadias, com a presença de moléculas de LDL-c pequenas e densas e níveis de Lipoproteína(a) aumentados (31,32). A severidade da dislipidemia parece variar conforme o fenótipo da doença, sendo mais significativa em pacientes com a forma clássica da doença em comparação ao fenótipo ovulatório do PCOS. (32).

Estudos angiográficos buscando marcadores precoces de disfunção endotelial em mulheres com PCOS, demonstram um aumento na espessura média intimal de carótidas através de ultrasonografia e aumento do índice de cálcio coronariano determinado por tomografia computadorizada (37,38). Especula-se ainda, que pacientes com PCOS, quando comparadas a mulheres saudáveis de mesma idade, apresentam uma menor elasticidade venosa, avaliada através de estudo de dilatação mediada por fluxo, independente de obesidade (39).

Diversos estudos têm demonstrado que mulheres jovens com PCOS apresentam elevação de pressão arterial sistêmica em relação a mulheres sem a síndrome, com maior risco de evolução precoce para hipertensão (33, 34, 35, 36). Apesar de alguns autores relacionarem esta elevação da pressão arterial à resistência insulínica (33) ou obesidade (35), frequentemente presentes nestas mulheres, um estudo demonstrou uma correlação com hiperandrogenemia, independente de resistência insulínica, obesidade ou dislipidemia (34).

Associação entre PCOS e DM2 tem sido descrita em diversos estudos, inclusive pela International Diabetes Federation (IDF) (26). Estudos em adolescentes demonstram que a presença de PCOS está relacionada a uma maior incidência de intolerância à glicose, DM2 e da síndrome metabólica, quando comparadas a meninas da mesma faixa etária sem a doença (27, 28). Em recente meta-análise, a PCOS foi associada a um risco de 2,5 e 4,5 vezes para intolerância à glicose e DM2, respectivamente, em relação a mulheres saudáveis (8), agravado por presença concomitante de obesidade, diabetes gestacional e doença familiar de DM. Recentemente, Moran e cols demonstraram que mulheres com PCOS divididas pela presença de critérios do NIH e não-NIH teriam escores de risco para DM similar, porém maiores do que controles, independente de idade e adiposidade (29).

A maior prevalência de alterações metabólicas e fatores de risco cardiovasculares (CV) torna pacientes com PCOS um grupo em que medidas mais ativas de diagnóstico e prevenção de comorbidades devem ser tomadas. Por outro lado, evidências de que eventos cardiovasculares ocorram com maior frequência em mulheres com diagnóstico de PCOS ainda são escassas na literatura. Recentemente, uma meta-análise (21) objetivando avaliar o risco de doença arterial coronariana e Acidente Vascular Cerebral em mulheres com PCOS, inferiu que a presença de PCOS levaria a um aumento de duas vezes no risco de apresentar

tais eventos, quando comparadas a pacientes sem diagnóstico da síndrome, independente do IMC.

Alterações do sistema nervoso autonômico em pacientes com PCOS podem ser consideradas um achado precoce, pré-clínico, podendo contribuir para um potencial aumento de risco de doença cardiovascular nestas pacientes. Em estudo conduzido por Yldirir e cols. (13), pacientes com PCOS apresentaram um perfil de modulação autonômica cardíaca desfavorável, quando comparadas às mulheres sem a Síndrome. Neste estudo, os autores sugerem que as diferenças encontradas podem estar relacionadas às diferenças hormonais entre os dois grupos. Em outro estudo, avaliando a VFC (variabilidade da frequência cardíaca) durante 24 horas, Tekin e cols (30) observaram que a VFC encontra-se diminuída nas mulheres com PCOS, corroborando com os achados prévios da literatura. Entretanto, os estudos sobre este tema encontrados na literatura, não avaliaram as diferenças encontradas entre os diversos fenótipos de PCOS.

Apesar dos consistentes resultados demonstrando um aumento de fatores de risco cardiovascular em pacientes com diagnóstico de PCOS, ainda são escassos na literatura estudos que melhor avaliem as formas mais brandas da doença, bem como sua real associação às doenças cardiovasculares. Tendo em vista o papel do sistema nervoso autonômico no sistema cardiovascular e suas alterações precoces relacionadas a doenças cardiovasculares, o objetivo deste estudo foi avaliar a modulação do sistema nervoso autonômico em pacientes com diferentes fenótipos de PCOS, comparando-as com mulheres aparentemente saudáveis de mesma idade.

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Cardiac autonomic modulation in polycystic ovary syndrome: does the phenotype matter?

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Capsule

During sympathetic stimulation with a mental stress test, young patients with the classic PCOS phenotype showed lower heart rate variability response versus a healthy control group, after adjustment for age.

ABSTRACT

Objective: To assess whether heart rate variability (HRV) at rest and during sympathetic stimulation is disturbed in patients with different polycystic ovary syndrome (PCOS) phenotypes in comparison to healthy controls.

Design: Transversal study.

Setting: University hospital.

Patients: Thirty women with classic, anovulatory PCOS, 16 women with ovulatory PCOS, and 23 age-paired women with regular and proven ovulatory cycles.

Interventions: Anthropometric and hormonal evaluation and analysis of HRV (time and frequency domain HRV indices) at rest and following a mental stress test.

Main outcome measures: Difference between heart rate variability (HRV) components during rest and stress.

Results: Mean age was 22.80 ± 5.80 years in c-PCOS, 19.81 ± 6.43 years in ov-PCOS and 22.65 ± 5.89 years in controls. During mental stress, classic PCOS patients showed lower HRV response when compared to the control group, even after adjustment for BMI and age. When classic and ovulatory PCOS patients were considered together, total testosterone levels were inversely associated with the low frequency component, low frequency/high frequency ratio and the difference between high frequency response at rest and after the stress test.

Conclusions: Patients with the classic PCOS phenotype have an impaired autonomic modulation in response to sympathetic stimulation impaired autonomic modulation in response to sympathetic stimulation that is typical of considerably older women, or of advanced age.

Keywords: Heart rate variability; autonomic modulation; PCOS phenotype.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disease that affects women in their reproductive years. In addition to the typical association of hyperandrogenism and ovulatory dysfunction, an increase in cardiovascular risk factors has also been identified in several studies in patients with PCOS when compared to healthy women of the same age, including obesity (1, 2), lipid abnormalities (3, 4), insulin resistance (4, 5) and hypertension (6). Despite this evidence, an increased cardiovascular risk in PCOS still requires confirmation (7, 8).

Both metabolic and cardiovascular disorders are related to autonomic dysfunction, in which there is a compromise of blood pressure and heart rate, among others (9-12). Previous studies have shown that reductions in heart rate variability (HRV), a measure of the time variation between heart beats (R-R interval) (13), may occur in patients with the metabolic syndrome (10, 14) or diabetes mellitus (9) even in the absence of established cardiovascular disease. These findings suggest a possible link between HRV and insulin resistance or metabolic abnormalities.

While PCOS patients may have pronounced metabolic abnormalities, a few studies have shown slight impairment in HRV in these women. Yildirir et al. (15) found a significant increase in the low frequency (LF) component of the HRV spectrum and a decrease in the high frequency (HF) component in relation to the control group. Tekin et al. (16) showed a decrease in heart rate recovery and decreased 24-hour HRV measurement in patients with PCOS.

One aspect to consider, however, is that as a result of the inclusion of polycystic ovary appearance as a major criterion for the diagnosis of PCOS in 2003 (Rotterdam Consensus) (17), a broader spectrum of disease phenotypes has emerged, including milder forms of the

syndrome. These milder phenotypes may differ from the "classic" PCOS phenotype (patients with anovulatory cycles and hyperandrogenism) regarding the severity of insulin resistance and prevalence of cardiovascular risk factors (18-21).

We hypothesized that HRV might behave differently depending on PCOS phenotype. Therefore, the aim of this study was to assess HRV at rest and during sympathetic stimulation in patients with classic and ovulatory PCOS and in comparison with age-paired healthy controls.

MATERIALS AND METHODS

Subjects

This transversal study was carried out with women aged 15 to 33 years, enrolled not earlier than two years after menarche, recruited by advertisement between 2009 and 2011. The advertisement asked for participants with hirsutism plus irregular or regular menses (in order to select for PCOS, both phenotypes) and others without hirsutism and with regular menses (to be selected as controls). One hundred and forty women were enrolled, 48 were excluded (did not meet inclusion criteria) and 23 did not agree to interrupt oral contraceptive pill use or dropped out during the study. Among the remaining 69 participants, PCOS was diagnosed in 46 patients according to the Rotterdam criteria: 30 patients were classified as classic PCOS (c-PCOS) [biochemical and/or clinical hyperandrogenism and oligo/amenorrheic cycles (< 9 cycles/year), with or without polycystic ovary (PCO) appearance at ultrasound] and 16 were characterized as ovulatory PCOS (ov-PCOS) [hirsute women with normal androgen levels, regular, ovulatory cycles confirmed by luteal phase progesterone > 3.8 ng/mL, and PCO]. PCO was defined as ovarian volume greater than $10mm^3$ in at least one ovary. Hirsutism was defined as a modified Ferriman Gallwey score ≥ 8

(17). Twenty-three non-hirsute women with regular and proven ovulatory cycles (luteal phase progesterone > 3.8 ng/mL) were included in the study as a control group. None of the cases or controls had received any drugs known to interfere with hormonal levels (such as contraceptive pills, antiandrogens, metformin, fibrates, statins) for at least 3 months before the study. Women diagnosed with other hyperandrogenic disorders (nonclassic congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting neoplasms), thyroid disorders or hyperprolactinemia were excluded, according to previously reported (45, 46, 47). Also excluded were smokers, pregnant women, and patients with body mass index (BMI) > 38 kg/m^2, blood pressure (BP) > 160/100 mmHg, use of antihypertensive medications, and diabetes mellitus. The study protocol was approved by the local ethics committee (IRB-equivalent), and written informed consent was obtained from all subjects.

Study protocol

Anthropometric measures were taken, including weight, height and waist circumference (waist measured at the midpoint between the lower rib margin and the iliac crest) (22). Body mass index (BMI) was then calculated (current weight in kilograms divided by square of height in meters). Blood pressure was measured after a 10-minute rest, in the sitting position, with feet on the floor and the arm supported at heart level. Hormonal and metabolic assessments were made between the 2^{nd} and 10^{th} days of the menstrual cycle, or on any day if the patient was amenorrheic. All samples were obtained between 8 and 10 a.m. Blood samples were drawn after an overnight 12-hour fast for determination of plasma cholesterol, HDL-cholesterol and triglycerides. Glucose and insulin were measured before and 2 hours after the ingestion of a 75-g oral glucose load.

Blood samples were also drawn for measurement of sex hormone binding globulin (SHBG) and total testosterone (TT). Free androgen index (FAI) was estimated by dividing TT

(nmol/L) by SHBG (nmol/L) x 100 (23). Homeostasis model assessment index (HOMA index) was calculated by multiplying insulin (μIU/mL) by glucose (mmol/L) and dividing this product by 22.5 (24). LAP index for women was calculated using the formula [waist (cm) - 58] x triglyceride concentration (mmol/L), as previously reported (25, 26).

The level of physical activity was assessed by counting the daily number of steps with a digital pedometer (model BP 148, TECHLINE). The pedometer was configured and given to the patient with instructions for appropriate use of the device (27, 28).

Assays

Total cholesterol, HDL-cholesterol, triglycerides and glucose were determined by enzymatic colorimetric methods (Bayer 1650 Advia System, Mannheim, Germany). LDL cholesterol was estimated indirectly using the formula total cholesterol - HDL - triglycerides/5.

Total testosterone (TT) levels were measured by chemiluminescence (Siemens Advia Centaur XP, Deerfield, USA), with a sensitivity of 0.10 ng/mL and intra and inter assay coefficient of variation (CV) of 3.3 and 7.5% respectively. SHBG was measured by chemiluminescence (Immulite 2000 Siemens, Deerfield, USA), with a sensitivity of 0.02 nmol/L and intra and inter assay CV of 5.3 and 6.6% respectively. Plasma insulin levels were measured by electrochemiluminescence (Siemens Advia Centaur XP, Deerfield, USA), with a sensitivity of 0.50 U/mL and intra and inter assay CV of 2.8 and 2.1% respectively.

Heart rate variability (HRV)

For HRV analysis, participants were underwent a 30-minute ECG recording with a SEER Light digital recorder (GE Medical Systems Information Technologies, Milwaukee, WI). The recorded data were analyzed using a MARS 8000 analyzer (GE Medical Systems

Information Technologies, Milwaukee, WI) by an investigator (RSM) blinded to the patient's status (c-PCOS, ov-PCO or control). The following HRV indices were calculated in time and frequency domains using 5-minute segments as recommended by the European Society of Cardiology and North American Society of Pacing and Electrophysiology (29): the mean of all normal R-R intervals (Mean R-R), the root mean square of successive differences of normal adjacent R-R intervals (rMSSD), the percentage of successive differences between normal adjacent R-R intervals exceeding 50 ms (PNN50), low-frequency component (LF) (0.04-0.15 Hz), high-frequency component (HF) (0.15-0.5 Hz), and low-frequency/high-frequency ratio (LF/HF). Spectral components were expressed in normalized units (nu). The difference between HRV results obtained during rest and stress was calculated using the formula: value obtained for each variable during stress - value obtained at rest.

HRV testing was carried out in the morning, following a 2-h fast. Participants were instructed to abstain from caffeine, other products containing stimulants, alcoholic beverages and heavy exercise for 24 hours before the test. First, subjects rested quietly in the supine position, in a silent and semi-dark room for 20 minutes. After that, they were submitted to a Stroop color-word conflict test during 10 minutes. HRV was evaluated during the last five minutes of the rest and mental stress periods. In the color-word test, the subject is shown the printed names of colors on screens of a different color (e.g., the word "blue" on a red screen) and is asked to name the color of the screen rather than the word (30).

Statistical analysis

The sample size was estimated based on the study by Yildirir et al. (15), considering a power of 80% and alpha of 5%. To detect a difference of 10 ms^2 in HF between groups, 18 women would be required in each group.

Results are presented as means ± standard deviation (SD), or median and interquartile range. Non-parametric variables were organized by rank values to normalize distribution in order to allow comparisons between the groups by one-way analysis of variance/covariance (ANOVA/ANCOVA), followed by Bonferroni test. Comparisons of HRV indices were adjusted for BMI and age. The correlation between variables was performed by two-tailed Spearman rank correlation test considering the non-Gaussian distribution of variables. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 16, Chicago, IL, USA). Data were considered to be significant at $P < 0.05$.

RESULTS

The women participating in this study were young (c-PCOS 22.80 ± 5.80, ov-PCOS 19.81 ± 6.43 and Control group 22.65 ± 5.89 years). Most of subjects were Caucasians (93.4%, 93.7% and 96.7% for c-PCOS, ov-PCOS and controls, respectively). The remaining subjects were of mixed African and European ancestry. Table 1 summarizes the clinical, hormonal and metabolic profile of PCOS phenotypes and control groups. Age, number of steps per day, total and HDL-cholesterol were similar between the groups. C-PCOS patients had higher BMI, triglycerides and androgen levels compared with ov-PCOS patients and controls, even after adjustment of triglycerides and androgen levels for BMI.

While total and HDL-cholesterol values were similar between the groups, systolic and diastolic blood pressures were more elevated in c-PCOS patients compared to the control group. Waist circumference ($P = 0.001$), HOMA IR ($P = 0.006$) and LAP ($P = 0.001$), markers of insulin resistance, were also significantly higher in the c-PCOS group, but lost significance after adjustment for BMI.

Table 2 shows HRV indices at rest and after mental stress test for the three groups (c-PCOS, ov-PCOS and controls). Mental stress promoted a significant reduction in time domain

indices and HF component, and an increase in LF and LF/HF ratio in all groups, indicating that it was able to induce significant vagal withdrawal and sympathetic stimulation. At rest, no differences in HRV were found between groups. However, during sympathetic stimulation with the mental stress test, c-PCOS patients showed lower HRV response when compared to the control group, even after adjustment for BMI and age.

Differences in frequency domain HRV indices are shown in Figure 1. After stress, c-PCOS had lower ΔLF (Figure 1A) and ΔLF/HF values (Figure 1B) than controls. No difference between groups was found in ΔHF (Figure 1C). Ov-PCOS presented intermediate values between those found for c-PCOS and controls, but the difference was not significant.

Concerning time domain HRV indices, the c-PCOS group had lower mean of R-R intervals (MRR), PNN50 and RMSSD values during the stress test when compared to the control group (Figure 2A, B and C). The ov-PCOS phenotype presented intermediate time domain HRV values that did not differ significantly from those of the other two groups.

When all PCOS subjects were considered together (c-PCOS and ov-PCOS), total testosterone levels were inversely associated with LFnu (r = -0.264; *P* = 0.027), LF/HF (r = -0.280; *P* = 0.019) and Δ LF/HF index (r = -0.257; *P* = 0.032) during mental stress. No association was found between HOMA-IR or glucose levels and measures of HRV.

DISCUSSION

In the present study, the c-PCOS phenotype, but not the ov-PCOS phenotype, was associated with a reduction in cardiovascular autonomic modulation in a sample of young women without known cardiovascular disease. To our knowledge, this is the first study evaluating HRV indices both at rest and after sympathetic stimulation in different PCOS phenotypes.

Instantaneous control of heart rate depends on the interaction between the sympathetic and parasympathetic branches of the autonomic nervous system (31, 32). At rest, time and frequency domain indices mostly reflect vagal modulation, and no conclusions can be made on sympathetic modulation (33). During controlled sympathetic stimulation, a marked reduction in time domain HRV indices and HF spectral component is associated with vagal withdrawal, and the increase in the LF spectral component and L/H is associated with sympathetic modulation (34). Therefore, the assessment of HRV based on ECG recordings during rest and after mental stress allowed us to evaluate both the parasympathetic nervous system and sympathetic activation in young women with different PCOS phenotypes and normal controls.

Under these conditions, women with the more severe c-PCOS phenotype had an impaired sympathetic response to the mental stress test in comparison to the control group, as shown by a significantly lower increase in LFnu and LF/HF. Moreover, also in response to the mental stress test, the c-PCOS group showed a smaller reduction than that achieved at rest in predominantly vagal HRV indices, such as HFnu, MRR, PNN50 and rMSSD, compared to controls. Interestingly, the difference in sympathovagal control of HRV appeared only after the stress test, and not at rest, reflecting the fact that the young population in our sample was still healthy, with no influence of PCOS on autonomic modulation during rest conditions. However, during a situation requiring quick and effective autonomic modulation, the c-PCOS phenotype was associated with an impaired response. This autonomic imbalance implies a failure in the adaptation to stressors that may predispose to the development of a sustained perturbation of sympathovagal balance over time, possibly with higher risk of developing hypertension and insulin resistance. Impaired HRV in response to mental stress has also been reported in patients with diabetes mellitus (9) or recovering from myocardial infarction (32, 35), in whom the autonomic nervous system is compromised. This disturbed autonomic

modulation has been regarded as a predictor of cardiovascular events (11) and mortality (35, 36) in the general population.

A few previous studies have evaluated HRV in PCOS, showing impaired cardiac autonomic modulation at rest condition (15, 37) and during 24 hours (16) in women with PCOS in comparison to controls. However, those studies did not focus on a comparison between PCOS phenotypes. In contrast, the present study was designed to specifically evaluate the differences in HRV between anovulatory and ovulatory PCOS. Interestingly, ov-PCOS presented intermediate values that did not differ significantly in relation to either healthy controls or the c-PCOS phenotype group. While no previous studies are available concerning HRV in ov-PCOS, the present findings and the results of other studies on the characteristics of PCOS phenotypes (21, 38) support the hypothesis of a less severe clinical presentation for ovulatory PCOS, with an intermediate metabolic and reproductive profile, when compared to patients with the full blown syndrome and healthy women.

Because some studies have shown a worsening in HRV with aging (39, 40), this variable was adjusted for all analyses in the present study, even though the mean age of PCOS and control participants was similar. In addition, our PCOS patients were slightly younger (mean age of 21.3 years) than those included in other studies, in which the mean age ranged from 25 to 31 years (15, 16, 37). Therefore, we speculate that the differences between our study and previous reports in HRV at rest could be, at least in part, related to age differences. In this sense, one limitation of our study is the small size of the sample, which could not be stratified according to age. Further studies are thus needed in order to compare HRV at rest and after mental stress in different age classes.

Androgens have been related to cardiovascular risk factors in PCOS women (4). While no differences have been found in the magnitude of reactivity of women and man in response to the Stroop Test (48, 49) we found a negative and significant correlation between total

testosterone levels and frequency domain HRV indices during stress in PCOS women, suggesting that androgens may account for the blunted autonomic response in this special group of women, as also observed by others (15, 16). It is of note that androgen levels were measured by immunoassay with quality control standards. While it may be considered a limitation of the study, since liquid chromatography mass spectrometry has been proposed as the preferable assay for quantifying serum testosterone levels in women, the accuracy and low cost of well-chosen and maintained direct immunoassays still warrant the use of such assays to measure total testosterone in hyperandrogenic women. As expected, we found significant differences among control and PCOS groups.

There is evidence of an association between insulin resistance and cardiovascular risk reflected by changes in HRV in hyperinsulinemic and diabetic patients (41-43), regardless of age (44). In our study, the significantly higher levels of insulin resistance markers, such as waist, HOMA-IR and LAP, observed in the c-PCOS group compared to ov-PCOS and controls were lost after adjustment for BMI. This means that our young c-PCOS patients may not yet be severely insulin resistant, which could explain the absence of association between HOMA-IR or glucose levels and measures of HRV.

In conclusion, the results of the present study indicate that patients with the classic PCOS phenotype have an impaired autonomic modulation in response to sympathetic stimulation that is typical of considerably older women, or of advanced age. Prospective longitudinal studies are required to confirm the clinical relevance of these data.

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Figures

Table 1. Clinical, hormonal and metabolic features of patients with classic PCOS, ovulatory PCOS and controls

Variable	Classic PCOS (n = 30)	Ovulatory PCOS (n = 16)	Controls (n = 23)	*P*	*P**
Age (years)	22.80 ± 5.80	19.81 ± 6.43	22.65 ± 5.89	0.237	-
Body mass índex	31.47 ± 5.21[a]	24.47 ± 3.62[b]	27.18 ± 5.36[a,b]	0.001	-
Waist circumference (cm)	90.56 ± 12.89[a]	75.14 ± 8.70[b]	82.89 ± 11.32[a,b]	0.001	0.887
Systolic blood pressure (mmHg)	119.10 ± 12.44[a]	116.70 ± 14.89[a,b]	108.67 ± 10.59[b]	0.016	0.014
Diastolic blood pressure (mmHg)	78.62 ± 9.53[a]	73.80 ± 10.82[a,b]	70.48 ± 6.87[b]	0.009	0.027
Steps (mean/day)	6648 ± 4053	5871 ± 3989	6204 ± 3499	0.688	-
Total testosterone (ng/mL)	0.81 ± 0.25[a]	0.64 ± 0.20[b]	0.57 ± 0.15[b]	0.001	0.001
Free androgen índex	13.14 ± 6.38[a]	9.25 ± 6.74[b]	6.10 ± 3.87[b]	0.050	0.063
Total cholesterol (mg/dL)	181.60 ± 39.66	158.88 ± 25.68	176.95 ± 25.94	0.083	0.349
High density lipoprotein (mg/dL)	44.83 ± 14.55	51.31 ± 13.43	52.14 ± 10.19	0.098	0.558
Triglycerides (mg/dL)	119.27 ± 61.41[a]	86.56 ± 31.08[b]	79.10 ± 29.08[b]	0.004	0.028
LAP	37.24 (23.49-70.35)[a]	16.46 (6.56-24.33)[b]	21.59 (11.97-31.64)[b]	0.001	0.239
HOMA IR	3.67 (2.46-5.84)[a]	2.59 (1.22-3.34)[b]	2.94 (1.48-3.71)[b]	0.030	0.411

HOMA = homeostasis model assessment; LAP = lipid accumulation product; PCOS = polycystic ovary syndrome. Total testosterone reference range for women: 0.2-0.8 ng/mL.

Values are expressed as mean ± SD or median and 25-75 interquartile range.

Different superscript letters indicate statistical difference by ANOVA and Bonferroni test.

* *P* value adjusted for BMI

Table 2. Heart rate variability indices at rest and during stress test

	Classic PCOS (n = 30)			Ovulatory PCOS (n = 16)			Controls (n = 23)		
Variable	Rest	Stress	*P*	Rest	Stress	*P*	Resting	Stress	*P*
LFnu	0.45 (0.31-0.56)[a]	0.70 (0.61-0.80)[b]	0.001	0.35 (0.30-0.50)[a]	0.71 (0.61-0.85)[b,c]	0.001	0.41 (0.36-0.51)[a]	0.81 (0.74)[c]	0.001
HFnu	0.52 (0.40-0.65)[a]	0.25 (0.16-0.30)[b]	0.001	0.62 (0.48-0.67)[a]	0.24 (0.11-0.35)[b,c]	0.005	0.55 (0.47-0.59)[a]	0.17 (0.12-0.22)[c]	0.001
LF/HF	0.8 (0.50-1.35)[a]	2.8 (1.90-5.15)[b]	0.001	0.54 (0.42-1.05)[a]	3.05 (1.72-8.07)[b,c]	0.001	0.7 (0.60-1.10)[a]	4.9 (3.37-7.30)[c]	0.001
Mean RR (ms)	925 (862-990)[a]	669 (637-761)[b]	0.001	945 (888-981)[a]	652.5 (615-734)[b,c]	0.001	864 (823-929)[a]	629 (605-665)[c]	0.001
PNN50 (%)	33 (20-60)[a]	4 (2-16)[b]	0.001	45 (31-60)[a]	3.25 (0.25-10)[b,c]	0.001	25.25 (12-38)[a]	1.82 (0.53-8)[c]	0.001
rMSSD (ms)	54.55 (39-94)[a]	24 (20-40)[b]	0.001	66.16 (49-93)[a]	24 (16-31)[b,c]	0.001	45.23 (34-64)[a]	20 (16-29)[c]	0.001

Values are expressed as median and interquartile range: 25%-75%.

P = rest *versus* stress for each group (ANCOVA and Bonferroni test, adjusted for BMI and age).

a, b, c = different letters express significant difference ($P < 0.01$).

HFnu = high frequency normalized units; LFnu = low frequency normalized units; LF/HF = low frequency/high frequency ratio; Mean R-R = mean of all normal R-R intervals; PNN50 = percentage of successive differences between normal adjacent R-R intervals above 50 ms; rMSSD = root mean square of successive differences between adjacent R-R intervals.

Figure 1. Spectral analysis of HRV components in response to the mental stress test in c-PCOS, ov-PCOS and controls. 1A, delta of low-frequency component (LF) in normalized units; 1B, delta of low-frequency/high-frequency ratio; 1C, delta of high-frequency component (HF) in normalized units. Delta (Δ) is the difference between the results obtained in HRV during rest and stress.

* $P < 0.05$ by ANCOVA and Bonferroni tests (BMI and age as covariates).

Values are expressed as median and 25%-75% interquartile range.

Figure 1A

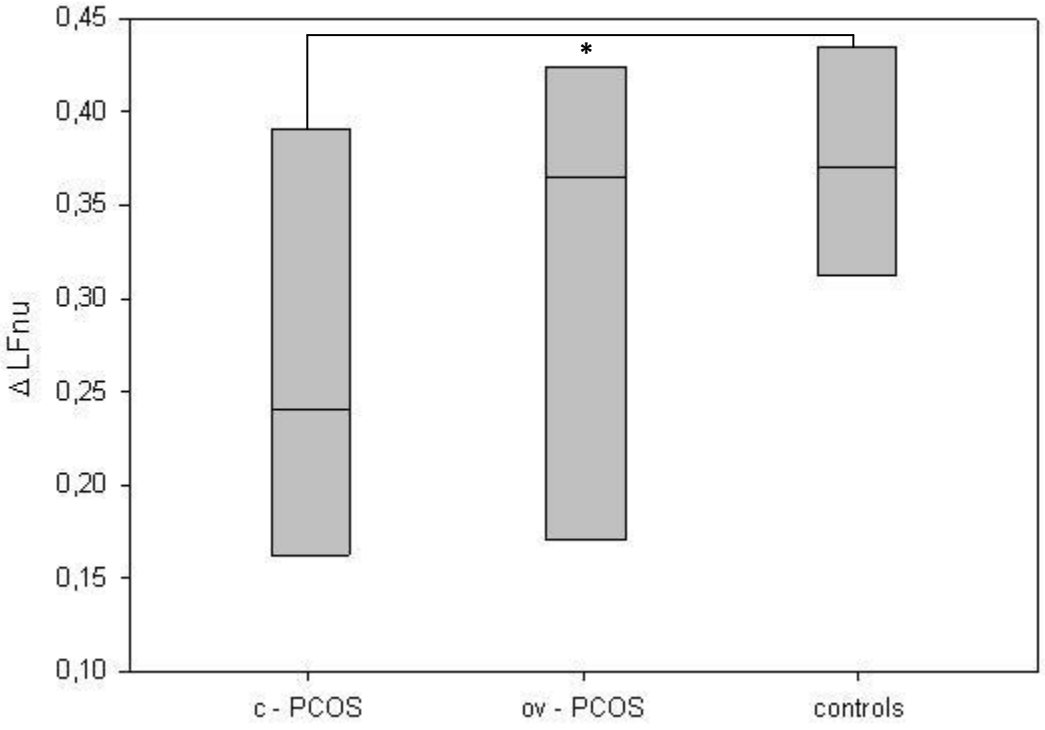


Figure 1B.

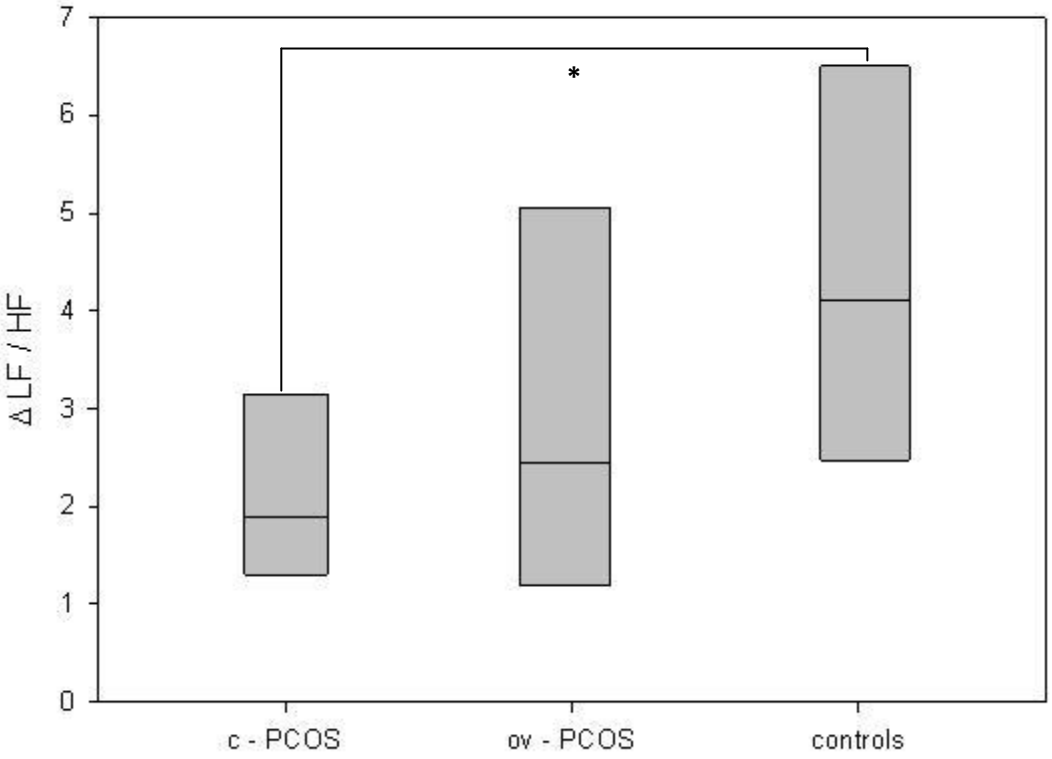


Figure 1C.

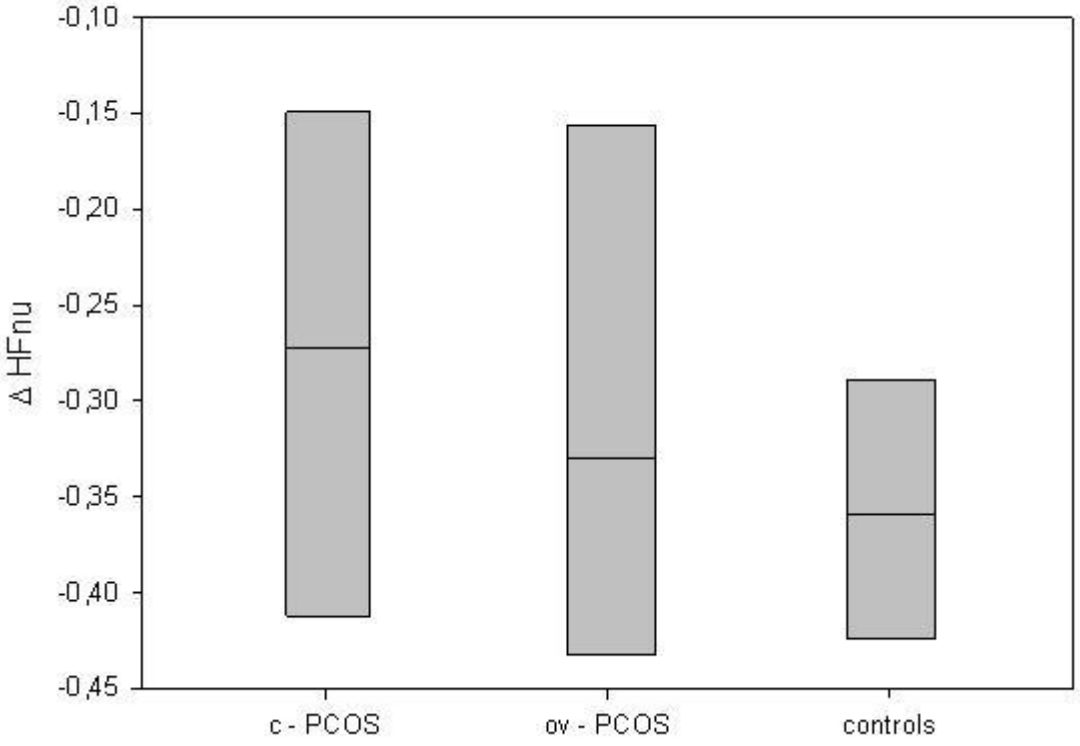


Figure 2. Time domain HRV components in response to mental stress test in c-PCOS, ov-PCOS and controls. 1A, normal R-R intervals; 1B, percent differences between normal adjacent R-R intervals exceeding 50 ms (PNN50); 1C, root mean square of successive differences of normal adjacent R-R intervals (rMSSD).

* $P < 0.05$ by ANCOVA and Bonferroni tests (BMI and age as covariates).

Values are expressed as median and 25%-75% interquartile range.

Figure 2A.

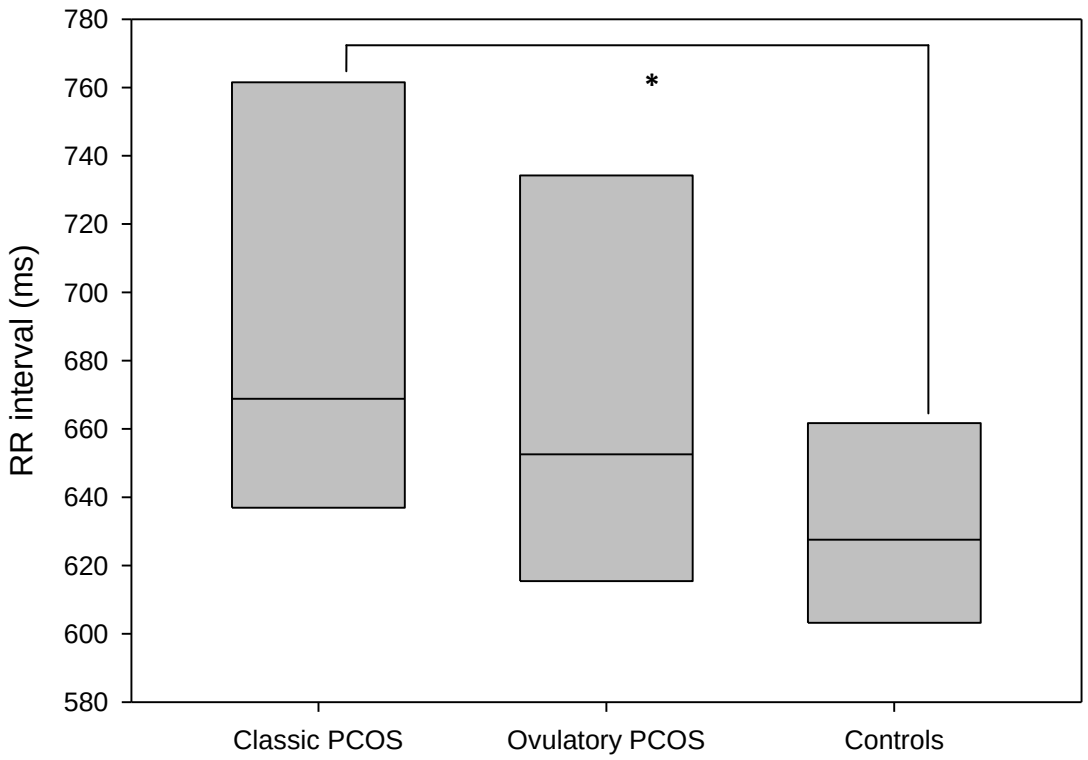


Figure 2B.

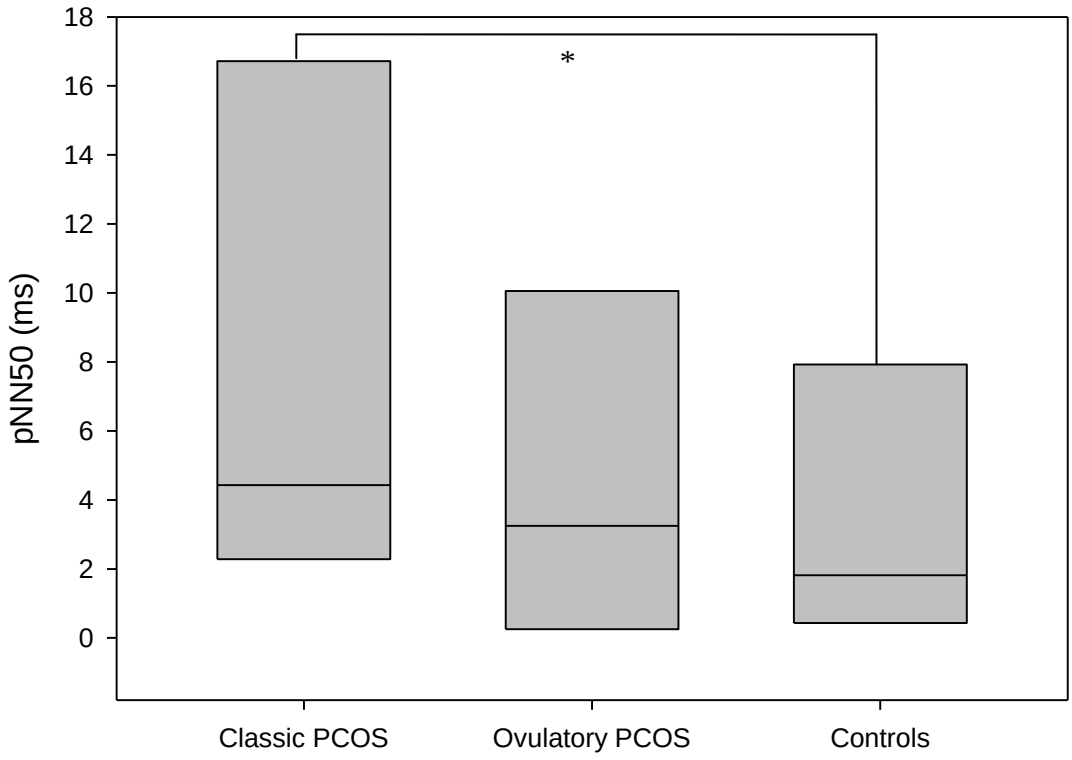
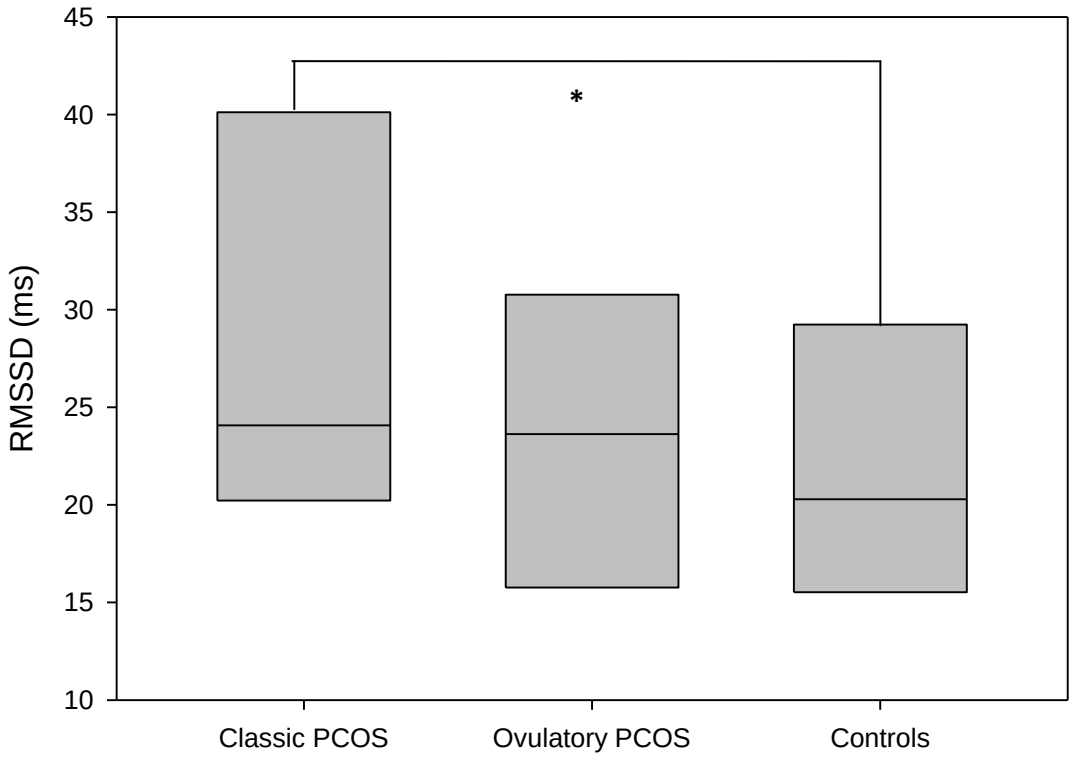

18
16
14
12
10
8
6
4
2
0
pNN50 (ms)
*
Classic PCOS
Ovulatory PCOS
Controls

Figure 2C.


45
40
35
30
25
20
15
10
RMSSD (ms)
*
Classic PCOS
Ovulatory PCOS
Controls

Abbreviations:

BMI	Body mass index
BP	Blood pressure
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
FAI	Free androgen index
HDL-C	High-density lipoprotein cholesterol
HF	High frequency
HFnu	High frequency normalized unit
HOMA	Homeostasis model assessment
HRV	Heart rate variability
IR	Insulin resistance
LAP	Lipid accumulation product
LDL-C	Low-density lipoprotein cholesterol
LF	Low frequency
LFnu	Low frequency normalized unit
LF/HF	Low-frequency/high-frequency ratio

c-PCOS	Classic polycystic ovary syndrome
ov-PCOS	Ovulatory polycystic ovary syndrome
PCO	Polycystic ovary
PCOS	Polycystic ovary syndrome
PNN50	Percentage of successive differences between normal adjacent RR intervals above 50 ms
rMSSD	Root mean square of successive differences of adjacent RR intervals
R-R	Mean of all normal RR intervals
SBP	Systolic blood pressure
SHBG	Sex hormone binding globulin
TT	Total testosterone
WC	Waist circumference
Δ	Delta = difference between the results obtained in HRV during rest and stress