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Associação entre modulação autonômica e androgênios endógenos em uma amostra de mulheres pós-menopáusicas

Roberta Fernandes Franz

Porto Alegre, 10 de abril 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.

Orientadora: Profª. Drª. Poli Mara Spritzer

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- Revisão: Associação entre modulação autonômica e androgênios endógenos em uma amostra de mulheres pós-menopáusicas

- Artigo original: Association between androgenicity and decreased heart rate variability in apparently healthy postmenopausal women: a cross-sectional study

SUMÁRIO

Introdução

O sistema nervoso autonômico (SNA) tem influência em diferentes situações fisiológicas e patológicas que afetam o sistema cardiovascular, o que desperta o interesse no conhecimento do papel do SNA nas doenças cardiovasculares. Embora a automaticidade cardíaca seja intrínseca, o ritmo cardíaco é amplamente determinado pelo SNA, que consiste na interação dos sistemas simpático e parassimpático (Lauer, 2009). A "perturbação do balanço autonômico", seja por diminuição da modulação vagal, aumento na ativação simpática ou a combinação de ambos, pode levar à doença cardiovascular (De Meersman et al, 2007).

A análise detalhada da flutuação da frequência cardíaca (FC) pode ser utilizada como um método indireto de avaliar o controle autonômico do coração. A variabilidade da frequência cardíaca (VFC) descreve oscilações dos intervalos entre batimentos cardíacos consecutivos (intervalos R-R) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, Circulation 1966). O aumento da VFC é um sinal de boa adaptação, indicando mecanismos autonômicos eficientes. Ao contrário, reduzida VFC sugere adaptação anormal do SNA (Pumprla et al, 2002; Acharya et al, 2006).

O conhecimento de que as flutuações da frequência cardíaca refletem a interação nos dois grandes componentes autonômicos veio a oferecer uma janela para o estudo do SNA a partir da variabilidade da frequência cardíaca (Fouad et al, 1984). Como ferramenta de pesquisa, a caracterização da VFC tem permitido um melhor entendimento da participação do SNA em diferentes situações fisiológicas e patológicas do sistema cardiovascular (Kleiger et al, 2005). O comportamento destas oscilações da

FC pode ser avaliado, utilizando-se diferentes métodos. Dos métodos lineares os índices são obtidos no domínio do tempo e no domínio da frequência (Pumprla et al, 2002).

O método linear que analisa o domínio do tempo mede cada intervalo RR normal (batimentos sinusais) durante determinado intervalo de tempo e, a partir disto, com base em métodos estatísticos ou geométricos calcula-se a dispersão em torno da média da FC. Os índices no domínio do tempo diferem entre si apenas na escolha da abordagem matemática para traduzir a dispersão dos intervalos RR em torno da média. Os índices estatísticos mais utilizados são: a) SDNN - desvio padrão de todos os intervalos RR normais gravados em um intervalo de tempo, expresso em ms; b) rMSSD - é a raiz quadrada da média do quadrado das diferenças entre intervalos RR normais adjacentes, em um intervalo de tempo, expresso em ms; c) pNN50 - representa a porcentagem dos intervalos RR adjacentes com diferença de duração maior que 50ms (Kleiger et al, 1991). O SDNN representa a atividade dos sistemas simpático e parassimpático, enquanto que rMSSD e pNN50 representam atividade parassimpática (Pumprla et al, 2002; Kleiger et al, 1991).

Os métodos no domínio da frequência conseguem identificar oscilações nos seguintes componentes de frequência: a) Alta frequência (HF): variações de 0,15 a 0,4 Hz correspondem à ação do parassimpático sobre o coração; b) Baixa frequência (LF): variações entre 0,04 e 0,15 Hz decorrentes da ação conjunta dos componentes vagal e simpático; c) Muito baixa frequência (VLF): pouco utilizado e explicação fisiológica não bem estabelecida; d) Relação LF/HF caracterizando o balanço simpato-vagal sobre o coração (Akselrod S et al, 1985). Para minimizar os efeitos das alterações da banda de VLF utiliza-se a normalização dos dados da análise espectral, dividindo um

componente (LF ou HF) subtraído do componente VLF, pela potência total e multiplicando por 100.

As medidas da VFC dos domínios do tempo e frequência são estreitamente relacionadas, podendo ser utilizadas indiferentemente (Pumprla et al, 2002; Bigger et al, 1992).

Embora o registro do ECG de 24 horas seja utilizado na maioria dos estudos que avaliam VFC, pesquisadores mostram resultados clinicamente relevantes com registros tão curtos quanto 5 minutos (Sloan et al, 1994; Pumprla et al, 2002; Schroeder et al, 2005). Estes geralmente são feitos sob condições controladas no repouso, porém, podem-se utilizar também estímulos como o teste de inclinação, uso de drogas, exercício físico, modificação postural, testes de estresse psicológico ou outras manobras selecionadas para ativar o sistema nervoso simpático (Kleiger et al, 2005; Delaney et al, 2000; Hamer et al, 2007). Estudos mostram que durante a posição supina existe uma predominância do sistema nervoso parassimpático. Durante modificação postural ou outro estímulo ocorre predomínio do SN simpático somado à retirada do SN parassimpático (Pumprla et al, 2002).

Um dos trabalhos mais citados na literatura e de grande impacto clínico no estudo da variabilidade da frequência cardíaca foi publicado por Kleiger, demonstrando que pacientes que apresentavam diminuição da VFC, medida pelo SDNN, após infarto do miocárdio, apresentavam maior mortalidade (Kleiger et al, 1987). No estudo da coorte de Framingham, a diminuição da VFC foi associada ao aumento de risco de eventos cardíacos em uma coorte de 2501 homens e mulheres com idade média de 52 e 54 anos, respectivamente, sem doença cardíaca aparente (Tsuji et al,1996). Este mesmo grupo demonstrou aumento de risco para mortalidade de todas as causas associada à

redução da variabilidade da frequência cardíaca em uma coorte de idosos (Tsuji et al, 1994). Muitos outros estudos identificam disfunção autonômica cardíaca como preditor de risco cardiovascular e mortalidade de causas cardíacas e não cardíacas (Moore et al, 2006; Lauer, 2009). Além disso, os índices de VFC encontram-se reduzidos em pacientes com doenças crônicas (Hildreth, 2012), diabete melito com neuropatia autonômica (Liao et al, 1995), síndrome metabólica (Liao et al, 1998).

Modificações autonômicas cardíacas são observadas durante o processo fisiológico de envelhecimento (Antelmi et al, 2004; Acharya et al, 2004; Stein et al, 2009) e a menopausa parece ter um impacto negativo na atividade autonômica, com diminuição da VFC (Earnest et al., 2008; Brockbank et al, 2000). Neste subgrupo de mulheres, estudos têm demonstrado associação negativa entre VFC e proporção de gordura corporal, níveis de pressão arterial e de lipídeos séricos (Kimura et al, 2006, Doncheva et al, 2003). Também associação de redução da variabilidade cardíaca com doença cardiovascular subclínica, identificada por maior extensão de calcificação nas artérias coronárias e na aorta (Gianaros et al, 2005).

A doença cardiovascular (DCV) continua sendo a principal causa de morte feminina (AHA, 2012), tendo um acréscimo exponencial destes índices após a menopausa (Assmann et al., 1999), o que disperta o interesse para o impacto dos hormônios sexuais na incidência da DCV. Lambrinoudaki demonstrou elevada prevalência de doença cardiovascular subclínica em mulheres jovens, na pós-menopausa recente, com baixo a médio *scores* de risco clínico (Heartscore) (Lambrinoudaki et al, 2011).

Androgênios tem sido relacionados à fatores de risco cardiovascular (Coviello et al, 2006; Wild et al, 2010; Wiltgen et al, 2010). Mulheres na menacme com síndrome dos

ovários policísticos (PCOS) apresentam maiores índices de obesidade quando comparada com controles (Wiltgen et al, 2010), assim como maior adiposidade visceral (Lord et al, 2006), maior resistência insulínica (Goodarzi et al, 2005) e dislipidemia (Wild et al, 2011). Recentemente, estudos pequenos tem sugerido associação de PCOS com doença arterial subclínica (Soares et al, 2009), contudo mais estudos são necessários para inferir desfechos cardivasculares neste grupo de mulheres (Fauser et al, 2011).

Na pós-menopausa também tem sido demonstrada a associação positiva entre níveis de androgênios e fatores de risco cardiovasculares, tais como obesidade, dislipidemia, resistência insulínica e marcadores inflamatórios (Patel et al, 2009; Maturana et al, 2008; Maturana et al, 2002). Mais recentemente, Creatsa demonstrou associação entre androgênios endógenos e doença arterial subclínica na pós-menopausa recente (Creatsa et al, 2011) e Maturana et al encontraram associação entre índices de androgênios livres e disfunção endotelial precoce (Maturana et al, 2010).

A associação da menopausa e androgênios endógenos com fatores de risco cardiovascular e aumento de doença cardiovascular subclínica vem sendo evidenciada em diferentes estudos. Por outro lado, a redução na modulação autonômica tem sido associada com estes mesmos fatores de risco cardiovascular bem como o processo de envelhecimento e o *status* pós-menopausal parecem estar relacionados com a atividade autonômica através da diminuição da VFC.

A elevada prevalência de DCV após a menopausa estimula a busca por marcadores que possam identificar uma subpopulação de mulheres sob maior risco de eventos cardiovasculares. Estudos prévios sugerem que androgênios endógenos mais próximos do limite superior podem fazer parte de um perfil de risco cardiovascular nesta

população. Contudo a relação entre níveis circulantes de androgênios endógenos e modulação autonômica cardíaca em mulheres na pós-menopausa ainda não foi elucidada. Portanto, o objetivo deste estudo foi avaliar se androgênios endógenos estão associados com alteração na modulação autonômica em mulheres aparentemente saudáveis na pós-menopausa.

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Association between androgenicity and decreased heart rate variability in apparently healthy postmenopausal women: a cross-sectional study

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Abstract

Endogenous androgens appear to be associated with endothelial dysfunction and increased cardiovascular risk in the postmenopause. Also, the menopause entails a reduction in cardiac autonomic modulation assessed by heart rate variability (HRV). We performed a cross-sectional study to verify whether the free androgen index (FAI) affected HRV in 87 apparently healthy postmenopausal women. After clinical and laboratory evaluations, time and frequency domain HRV indices were determined at rest and during sympathetic stimulation (mental test). Patients were stratified according to FAI≤2.5 or >2.5. Mean age was 55 (±5) years. Median time since menopause was 6 (3-10) years. Mean body mass index (BMI) was 27.12±4.49 kg/m². Metabolic syndrome was diagnosed in 26 (29.5%) participants. Mean systolic and diastolic pressure was 128.64±17.98 mmHg and 78.89±10.32 mmHg, respectively; 38 participants (43.6%) had hypertension. Women with FAI>2.5 had higher BMI, waist circumference, fasting insulin, HOMA, triglycerides, and total testosterone (TT). These differences disappeared after adjustment for BMI, except for TT. Presence of metabolic syndrome, history of smoking, hypertension and use of anti-hypertensive drugs were similar in both groups. Mental stress promoted a reduction in time domain indices and HF component, and an increase in LF and LF/HF ratio in both groups, indicating the reliability of the mental stress test to induce vagal withdrawal and sympathetic stimulation. In women with FAI≤2.5, mean rMSSD (P=0.015) and PNN50 (P=0.005) were higher at rest as compared to women with FAI>2.5, after adjustment for BMI. In conclusion, endogenous androgens may be associated with decreased HRV in apparently healthy postmenopausal women.

Keywords: Postmenopause, free androgen index, cardiac autonomic modulation, cardiovascular risk.

Introduction

Although much progress has been made in the prevention and treatment of cardiovascular disease (CVD), it is still the leading cause of death among women worldwide (Roger et al. 2012). Women develop CVD typically after the menopausal transition and approximately 10 years later than men (Schenck-Gustafsson 2009). This has been interpreted as reflecting estrogen-mediated protection against atherogenesis (Mendelsohn and Karas 2005).

Recently, androgens have also been associated with cardiovascular risk (Creatsa et al. 2012; Maturana et al. 2008; Maturana et al. 2011). A retrospective analysis of the Women's Health Initiative Study has established that decreased levels of sex hormone-binding globulin (SHBG) and increased free testosterone are associated with the development of cardiovascular disease in postmenopausal (Rexrode et al. 2003) and perimenopausal women (Sutton-Tyrrell et al. 2005). Circulating testosterone has also been linked to subclinical atherosclerosis and arterial stiffness, independently of age, body mass index (BMI), lipids, and insulin resistance (IR) (Creatsa et al. 2012).

The involvement of the autonomic nervous system is considered crucial for the progression of various pathologies that affect the cardiovascular system, including myocardial infarction (Kleiger et al. 1987), congestive heart failure (Moore et al. 2006), and sudden death (Copie et al. 1996). In this context, the analysis of beat-to-beat heart rate variability (HRV) has been used as a noninvasive tool to evaluate cardiovascular autonomic regulation (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Reduced HRV reflects a reduction in cardiac autonomic regulation and is associated with a worse prognosis. Patients with decreased HRV one week after myocardial infarction have higher one-

year mortality than patients with higher HRV at the same time point (Kleiger et al. 1987).

Impaired autonomic modulation is related to adverse health effects (Kleiger et al. 2005; Liao et al. 1998; Tsuji et al. 1996), including subclinical atherosclerosis (Gianaros et al. 2005; Huikuri et al. 1999), and is considered as a predictor of cardiovascular risk and mortality from cardiac and noncardiac causes (Lauer 2009; Moore et al. 2006; Tsuji et al. 1994). A few studies have suggested a reduction in autonomic modulation associated with menopause and the ensuing hormonal changes (Brockbank et al. 2000; Earnest et al. 2008). However, the influence of androgenicity on cardiac autonomic modulation is unknown. Therefore, the aim of the present study was to assess whether free androgen index (FAI) is associated with disturbed HRV in apparently healthy postmenopausal women.

Methods

Patients

This cross-sectional study was carried out with women consulting for climacteric symptoms at the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil. Furthermore, volunteers were recruited by advertisement in a local newspaper and radio station. Inclusion criteria were as follows: 1) menopause, defined as last menstrual period at least 1 year before the beginning of the study plus follicle stimulating hormone (FSH) levels higher than 35 IU/L; 2) age between 45 and 65 years; 3) no use of hormonal therapy in the past 3 months; and 4) no use of beta-blocker therapy. Diabetic patients, patients with prior diagnosis of heart disease, and current smokers were excluded. Eighty-seven postmenopausal women fulfilling all the inclusion criteria were consecutively enrolled in the study. The study protocol was approved by the local Ethics Committee, and written informed consent was obtained from every subject.

Study protocol

Anthropometric measurements included body weight, height, waist circumference (waist measured at the midpoint between the lower rib margin and the iliac crest), and BMI (current measured weight in kilograms divided by height in square meters) (Toscani et al. 2007). Blood pressure was measured in the sitting position with feet on the floor and the arm supported at heart level after a 10-minute rest. Two measurements were performed, with an interval of 10 minutes, using automatic blood pressure monitor HEM-742INT OMRON. Hypertension was defined as systolic blood

pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current use of antihypertensive drugs (Chobanian et al. 2003). FSH, estradiol, total testosterone (TT), SHBG, total and high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and insulin were also determined using the fasting blood sample. All samples were obtained between 8 AM and 10 AM. Patients were stratified by FAI according to a cut-off point determined in a previous study (Maturana et al. 2010).

The presence of cardiovascular risk factors and frequency of metabolic syndrome were defined in accordance with the Joint Scientific Statement (Alberti et al. 2009).

Assays

Total cholesterol, HDL cholesterol and triglycerides were determined by colorimetric-enzymatic methods (Bayer 1800 Advia System), with intra and interassay coefficients of variation (CVs) <3%. Glucose was determined by hexokinase method (Advia 1800) with intra-assay CV <3.4% and interassay CV <2.1%. Low-density lipoprotein (LDL) cholesterol was determined indirectly using the Friedewald formula LDL = total cholesterol − HDL − triglycerides/5. FSH was measured by chemiluminescence immunoassay (Centaur XP), with intra and interassay coefficients of variation (CVs) of 2.9% and 2.7%, respectively. The sensitivity of the assays was 0.3 IU/L for FSH. TT levels were measured using a chemiluminescence immunoassay (Centaur XP) with sensitivity of 10 ng/mL and intra and interassay CVs of 3.3% and 7.5%, respectively. Sex hormone-binding globulin was measured by chemiluminescence enzyme immunoassay (Immulite 2000), with an assay sensitivity of

0.02 nmol/L and intra- and interassay CVs of 5.3% and 6.6%, respectively. Serum insulin levels were measured using chemiluminescent immunoassays (Centaur XP), with a sensitivity of 0.200 μIU/mL and intra- and interassay CVs of 2.0% and 4.3%, respectively. FAI was estimated by dividing TT (in nanomoles per liter) by SHBG (in nanomoles per liter) × 100. Homeostatic model assessment (HOMA) was calculated by multiplying insulin (uIU/ml) by glucose (mmol/l) and dividing this product by 22.5, as previously described (Matthews et al. 1985; Wiltgen et al. 2009).

Heart rate variability (HRV)

For HRV analysis, participants were submitted to 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 blinded to the patient's status (RSM). The following HRV indices were calculated in time and in frequency domains using 5-minute segments as recommended by the European Society of Cardiology and North American Society of Pacing and Electrophysiology (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996): 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).

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 instructed to stand up and were submitted to a Stroop color-word conflict test during 10 minutes. HRV was evaluated during the last 5-minute periods of rest and mental stress. In the color-word test, the subject is shown the printed names of colors in conflicting screen colors (e.g., the word "blue" in red screen) and is asked to name the color of the screen rather than the word (MacLeod 1991).

Statistical analysis

Sample size was estimated based on study published by Conrad P. Earnest (Earnest et al, 2008). Inferred that to detect the difference of 5 ms SDNN (standard deviation of RR intervals), whereas 80% power and alpha error of 5% will require 112 patients.

Study outcomes included HVR indices at rest and during mental stress, according to FAI index $\geq$ or $<$ 2.5. Results are expressed as means ± SD or median and interquartile range. Comparisons between the two group means were analyzed by Student t test; comparisons between median values were analyzed with the Mann-Whitney U test. Comparisons of HRV indices were adjusted for BMI (partial regression). Spearman rank or Pearson correlation coefficient was calculated between variables using a 2-tailed significance test for variables with a Gaussian or non-Gaussian distribution, respectively. Comparisons between ratios were carried out using

the χ^2 test. FAI and HOMA results were log-transformed for statistical analysis (multiple regression) and back-transformed for data presentation. All analyses were performed using the Statistical Package for the Social Sciences 16 (SPSS, Chicago, IL, USA). Data were considered to be significant at $P < 0.05$.

Results

Out of 107 volunteers, 20 were excluded (five with diabetes, two with hyperthyroidism, three with untreated hypothyroidism, one with cardiac surgery, two with breast cancer, three using beta blockers, one with oophorectomy, two dropouts, and one had many artifacts in ECG recording). Thus, 87 were enrolled. The mean age of participants was 55 (± 5) years. Age at menopause was 48 (± 3) years and the median time since menopause was 6 (3-10) years. Out of 87 participants, 82 (93.2%) were of European ancestry and 6 (6.8%) were of mixed African and European ancestry. The mean BMI was 27.12± 4.49 kg/m². Metabolic syndrome was diagnosed in 26 (29.5%) of patients. The mean systolic pressure was 128.64 ± 17.98 mmHg and mean diastolic pressure was 78.89 ± 10.32 mmHg and 38 patients (43.6%) had hypertension.

Table 1 presents the distribution of anthropometric, hormonal and metabolic variables according to the FAI cut-off point of 2.5 (Maturana et al. 2010). Both groups (FAI ≤ 2.5 or > 2.5) were similar regarding age and time since menopause. Women with FAI > 2.5 had higher BMI, waist circumference, fasting insulin, HOMA, triglycerides, and TT. These differences disappeared after adjustment for BMI, except for TT, which remained significantly higher in the group with FAI > 2.5. Presence of the metabolic syndrome (12 [13.8%] vs. 14 [16.1%], $P = 0.590$), history of smoking (19 [21.8%] vs. 13 [14.9%], $P = 0.210$), hypertension (21 [24.2%] vs. 17 [19.5%], $P = 0.441$) and use of anti-hypertensive drugs were similar in the groups (9 [10.34%] vs. 10 [11.49%], $P =$

0.708). Two participants in the FAI ≤ 2.5 group were using statin, and one was using aspirin.

Table 2 shows HRV indices at rest and after postural change and the mental stress test for FAI ≤ 2.5 and FAI > 2.5. Stress promoted a significant reduction in time domain indices and HF component, and an increase in LF and LF/HF ratio in both groups, indicating the reliability of the mental stress test in inducing vagal withdrawal and sympathetic stimulation.

BMI-adjusted time domain HRV indices are shown in Figure 1. Mean rMSSD and PNN50 values of women with FAI ≤ 2.5 were higher at rest when compared with those with FAI > 2.5 but were similar between groups during sympathetic stimulation with mental stress (Figure 1A and 1B). BMI-adjusted mean R-R values did not differ significantly between the groups.

Concerning frequency-domain analysis, no differences were found in BMI-adjusted data for the comparison of FAI groups (FAI ≤ 2.5 vs. FAI > 2.5), low frequency normalized units (LFnu) at rest (0.65 [0.41-1.27] vs. 0.64 [0.50-1.58], *P* = 0.466), LFnu during stress (0.84 [0.75-2.19] vs. 0.91 [0.79-1.85], *P* = 0.771), high frequency normalized units (HFnu) at rest (0.69 [0.55-1.53] vs. 0.57 [0.42-1.42], *P* = 0.254), HFnu during stress (0.31 [0.17-0.94] vs. 0.25 [0.12-0.95], *P* = 0.291), LF/HF at rest (0.78 [0.63-1.17] vs. 1.17 [0.80-1.55], *P* = 0.138), LF/HF during stress (2.69 [1.97-4.67] vs. 3.07 [2.06-6.77], *P* = 0.269).

Discussion

In the present study, androgenicity was associated with a reduction in cardiovascular autonomic modulation in an apparently healthy sample of postmenopausal women without known CAD. To our knowledge, this is the first report of such an association.

Assessment of HRV based on ECG recordings during rest and after ~~mental~~ stress (Delaney and Brodie 2000; Renaud and Blondin 1997) allowed us to evaluate both the parasympathetic nervous system and sympathetic activation in a group of postmenopausal women stratified by FAI, an index of endogenous androgens. This procedure was found to be a sensitive tool to detect subtle and short-term changes in sympathovagal balance.

Our patients were stratified by FAI using a cut-off point of 2.5. This value was chosen based on a previous study in which venous endothelial function was assessed in a carefully selected sample of healthy postmenopausal women, not presenting high blood pressure, IR or metabolic comorbidities (Maturana et al. 2010). In that study, FAI > 2.5 was associated with reduced vasodilatation response to acetylcholine, an early marker of endothelial dysfunction, even in the absence of known cardiovascular risk factors. In the present sample, participants with FAI > 2.5 had higher BMI, HOMA-IR and triglycerides, in agreement with another study from our group (Maturana et al. 2011) in which we observed a relationship between endogenous androgens and obesity, dyslipidemia and IR in postmenopause. Because metabolic parameters such as IR and obesity are known to negatively affect autonomic modulation, HRV analyses were controlled for BMI.

Evidence suggests that androgenicity is associated with obesity, dyslipidemia, IR and inflammatory markers (Maturana et al. 2008; Mesch et al. 2008; Sutton-Tyrrell et al. 2005). Androgens have been related to cardiovascular risk factors in women during both reproductive years (Coviello et al. 2006; Wild et al. 2010; Wiltgen and Spritzer 2010) and postmenopause (Maturana and Spritzer 2002; Patel et al. 2009). Creatsa et al. have reported an association between endogenous androgen levels and subclinical arterial disease in recently postmenopausal women (2012). We have also recently found an association between endogenous androgens and a state of attenuated endothelial-dependent response, indicating early endothelial dysfunction (Maturana et al. 2010). Therefore, elevated mean circulating androgen concentration is increasingly regarded as part of the risk profile related to CVD in postmenopausal women.

Autonomic changes in cardiovascular control have been observed with aging (Acharya et al. 2004; Antelmi et al. 2004; Ribeiro et al. 2001). These changes are related to a disturbed autonomic balance, in which there is either a decrease in vagal modulation or an increase in sympathetic modulation, or else a combination of both (De Meersman and Stein 2007). Menopause has been described as presenting a negative impact on autonomic activity by decreasing HRV (Brockbank et al. 2000; Earnest et al. 2008).

Furthermore, analysis of HRV has been used to study autonomic function and/or to estimate risk in a wide variety of cardiac and noncardiac disorders (Kleiger et al. 2005). Decreased HRV has been regarded as a predictor of mortality after acute myocardial infarction (Kleiger et al. 1987; Zuanetti et al. 1996), chronic heart failure (Moore et al. 2006) and advanced cancer (Fadul et al. 2010; Walsh and Nelson 2002). In addition, altered HRV has also been associated with chronic disease (Hildreth 2011),

diabetes mellitus (Liao et al. 1995), metabolic syndrome (Liao et al. 1998), and with more extensive calcification in the coronary arteries and in the aorta (Gianaros et al. 2005). However, no study has directly examined the relationship between endogenous androgens and HRV in an apparently healthy postmenopausal population.

In the present study, participants were stratified according to FAI (below or above cut off 2.5), with both groups having similar age and time since menopause. Interestingly, after BMI adjustment, subjects with FAI > 2.5 showed significantly lower time-domain HRV indices (rMSSD and pNN50) at rest, indicating a reduction in vagal activity. It is important to note that, in the presence of normal sinus rhythm and normal AV-nodal function, rMSSD and PNN50 quantify parasympathetic modulation of normal R-R intervals (Polanczyk et al. 1998). It is also important to highlight that we selected apparently healthy participants, and thus the observed reduced vagal modulation in the group with higher FAI may be regarded as a preclinical sign.

No differences could be detected between the two groups in HRV indices during stress. This may be explained, at least in part, by the older age of participants, in whom the increased sympathetic activity at rest might have prevented further increase in sympathetic modulation during mental stress in both groups. Perini et al, studying autonomic response to sympathetic stimulation with orthostatic load, observed that older subjects failed to increase sympathetic modulation and vagal withdrawal during stress compared to younger subjects (Perini et al. 2000). Most of the women in our study were recently postmenopausal, and both groups responded to stress with some degree of vagal withdrawal and sympathetic predominance, but perhaps not as strongly as observed in younger women. That might have prevented us from detecting differences in HRV indices between groups. Previous studies have reported diminished

sympathetic activation related to sympathetic stimulation with advancing age (Perini and Veicsteinas 2003).

Other studies have assessed HRV in women in association with aging or menopausal status. In a large cohort, 653 asymptomatic patients underwent 24-hour electrocardiographic recording. Time-domain indices decreased with age, reflecting reduced autonomic modulation (Antelmi et al. 2004). In another study, comparing premenopausal and postmenopausal women, Brockbank et al. showed that the main reduction in HRV starts after 2 years of menopause (2000).

One limitation of the present study is its cross-sectional design, which can only establish an association between endogenous androgens and HRV indices, but does not determine causality. Further longitudinal and cohort studies are needed to this end.

Conclusions

Our findings show that participants with higher endogenous androgens present a worse metabolic profile and lower cardiac autonomic modulation, suggesting that androgenicity may be associated with decreased HRV in apparently healthy postmenopausal women.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Ethical standards

This experiment complies with the current Brazilian laws applicable to research studies.

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Abbreviations:

BMI	Body mass index
CVD	Cardiovascular disease
FAI	Free androgen index
FSH	Hormone follicle stimulating
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
LDL-C	Low-density lipoprotein cholesterol
LF	Low frequency
LFnu	Low frequency normalized unit
LF/HF	Low-frequency/high-frequency ratio
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
SHBG	Sex hormone binding globulin
TT	Total testosterone

Table 1 Distribution of anthropometric, hormonal and metabolic variables according to free androgen index

Variable	FAI ≤ 2.5 (n = 44)	FAI > 2.5 (n = 43)	*P*	*P (age and BMI adjusted)*
Age (years)	56.23 ± 5.26	54.79 ± 4.98	0.194	-
Time since menopause*(months)	84 (36-120)	60 (29-120)	0.230	-
BMI (kg/m^2)	26.04 ± 4.10	28.31 ± 4.64	**0.018**	-
Waist circumference (cm)	83.70 ± 9.88	90.06 ± 12.32	**0.010**	**0.257**
Fasting glucose (mg/dL)	92.02 ± 9.02	93.86 ± 7.49	0.300	0.971
Fasting insulin (µIU/mL)*	7.66 (5,63-10.09)	10.62 (7.18-15.48)	**0.004**	**0.749**
HOMA-IR*	1.78 (1.23-2.26)	2.57 (1.58-3.57)	**0.007**	**0.547**
Total cholesterol (mg/dL)	212.25 ± 31.62	218.26 ± 36.20	0.410	0.261
HDL-C (mg/dL)	55.02 ± 12.23	53.39 ± 12.79	0.550	0.842
Triglycerides (mg/dL)*	81 (65-116.75)	112 (76-143)	**0.026**	**0.047**
Testosterone (pg/mL)	0.26 ± 0.12	0.49 ± 0.16	**0.001**	**0.001**
SHBG nmol/L	63.40 ± 28.08	38.46 ± 15.73	**0.001**	**0.001**
FAI*	1.51 (1.04-1.78)	4.03 (2.85-7.21)	**0.001**	**0.001**

Student t test (mean ± SD) or *Mann-Whitney U test (median and interquartile range: 25%-75%).

BMI body mass index, *FAI* free androgen index, *HOMA-IR* homeostasis model assessment – insulin resistance, *HDL-C* high-density lipoprotein cholesterol, *SHBG* sex hormone binding globulin

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

	FAI ≤ 2.5 (n = 43)			FAI > 2.5 (n = 44)		
Variable	Rest	Stress	*P*	Rest	Stress	*P*
LFnu*	0.65 (0.41-1.27)	0.84 (0.75-2.19)	0.001	0.64 (0.49-1.58)	0.91 (0.79-1.86)	0.001
HFnu*	0.69 (0.55-1.53)	0.31 (0.17-0.94)	0.001	0.57 (0.42-1.42)	0.25 (0.12-0.95)	0.001
LF/HF*	0.79 (0.63-1.17)	2.69 (1.97-4.67)	0.001	1.18 (0.81-1.55)	3.08 (2.06-6.78)	0.001
Mean R-R (ms)	963.52 ± 121.99	729.79 ± 115.44	0.001	911.21 ± 112.27	727.75 ± 90.27	0.001
rMSSD* (ms)	31.10(20.43-43.56)	15.47(12.26-19.75)	0.001	22.01(17.43-30.49)	13.90(11.86-17.89)	0.001
PNN50* (%)	9.81 (0.92-21.92)	0.44 (0.0-1.46)	0.001	1.66 (0.52-7.17)	0.0 (0.0-1.47)	0.001

Student t test (mean ± SD) or *Mann-Whitney U test (median and interquartile range: 25%-75%).

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

Figure legend:

Fig. 1 BMI and age-adjusted HRV indices at rest and during mental stress test in postmenopausal women according to FAI ≤ 2.5 or FAI > 2.5. A) median rMSSD and B) median PNN50