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# BMJ Open

## WOMEN WITH POLYCYSTIC OVARY SYNDROME PRESENT IMPAIRED CARDIAC AUTONOMIC FUNCTION AT A LATE FERTILE AGE: A LARGE PROSPECTIVE COMMUNITY-BASED COHORT STUDY

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Manuscripts

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7 2 **AUTONOMIC FUNCTION AT A LATE FERTILE AGE: A LARGE PROSPECTIVE**  
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9 3 **COMMUNITY-BASED COHORT STUDY**  
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53 24 **Word count:** 3546  
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**ABSTRACT**

**Objectives:** Previous studies of women in their 20s and 30s have reported impaired autonomic function in women with polycystic ovary syndrome (PCOS). We aimed to study, for the first time, whether PCOS is associated with impaired cardiac autonomic function independent of metabolic and hormonal status in their late reproductive years.

**Design:** A prospective birth cohort study including 5,889 females born in 1966 and followed through the age of 46. At that age,  $n=3,706/5,123$  women (72%) answered to the postal questionnaires and  $n=3,280/5,123$  women (64%) participated to the clinical examination.

**Setting:** General community.

**Participants:** Women presenting both oligoamenorrhea and hirsutism at age 31 ( $n=125$ ) or with formally diagnosed PCOS by age 46 ( $n=181$ ) and women without PCOS symptoms or diagnosis ( $n=1,577$ ).

**Interventions:** None.

**Primary and secondary outcome measures:** Heart rate variability (HRV) parameters: (rMSSD), spectral power densities (LF: low frequency, 0.04-0.15 Hz, and HF: high frequency, 0.15-0.40 Hz), and baroreflex sensitivity (BRS).

**Results:** We found that parasympathetic activity (assessed by root mean square of successive R–R differences [rMSSD]: 19.5 [12.4; 31.9] vs. 24.3 [16.1; 34.8] ms,  $P=0.004$  and high frequency [HF]: 172 [75; 399] vs. 261 [112; 565]ms<sup>2</sup>,  $P=0.002$ ) and baroreflex sensitivity ( $6.13\pm 3.12$  vs.  $6.99\pm 3.52$  ms/mmHg,  $P=0.036$ ) were lower in women with PCOS compared with controls. However, in the multivariate regression analysis, PCOS, body mass index, and free androgen index did not significantly associate with rMSSD, whereas blood pressure, insulin resistance, and triglycerides did.

**Conclusions:** We report here for the first time that late reproductive-aged women with PCOS display impaired cardiac autonomic function manifested as a decreased vagal activity. Metabolic status,

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3 50 rather than hyperandrogenemia and PCOS *per se*, was the strongest contributing factor. Given the  
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7 51 link between cardiac morbidity and impaired autonomic function, the findings underline the  
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9  
10 52 importance of screening and treating metabolic abnormalities early on in women with PCOS.  
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13 53 **Keywords:** PCOS, heart rate, heart rate variability, obesity, metabolism  
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19 55 **Article Summary section**  
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- 21 56
- 22 • This is the first study to investigate the cardiac autonomic function of late reproductive aged  
23  
24 57 women
  - 25  
26  
27 58 • This study provides by-far the largest study population compared to the previous studies in  
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31 59 cardiac autonomic function in women with PCOS
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34 60 • We were able to adjust for many confounding factors and to study the effect of metabolic  
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37 61 abnormalities
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40 62 • The study is limited by the lack of PCOS phenotypes
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44 63 • The study cannot be generalized with all ethnicities
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## 64 INTRODUCTION

65 Polycystic ovary syndrome (PCOS) is the most common endocrinopathy, affecting 6-18% of women  
66 of reproductive age and characterized by irregular menstruation, clinical or biochemical  
67 hyperandrogenism, and polycystic ovaries.[1-3] Women with PCOS are commonly overweight or  
68 obese and typically present with insulin resistance, hyperinsulinemia, increased blood pressure,  
69 dyslipidemia, metabolic syndrome, and obstructive sleep apnea,[2] all of which are associated with  
70 impaired cardiac autonomic function.[4-6] In the general population, dysregulation of cardiac  
71 autonomic function has been associated with increased risk of many major global public health  
72 problems, such as depression,[7] anxiety,[8] hypertension, diabetes, cardiovascular diseases, and  
73 mortality.[9] Therefore, it is not surprising that women with PCOS have been shown to present with  
74 impaired cardiac autonomic function, reduced parasympathetic (vagal) activity,[10-12] and increased  
75 sympathetic nervous system activity.[13] Investigators have used various methods, such as  
76 microneurography, measurement of sympathetic skin responses, heart rate (HR) variability (HRV),  
77 HR recovery, and noradrenaline spill over measurement. Of these methods, the measurement of  
78 HRV, i.e., variations in the time intervals between consecutive heartbeats, is an easy, effective, and  
79 well-established objective and non-invasive method utilizing standard electrocardiogram data that is  
80 mathematically analysed.[14]

81 During recent years it has become an enigma whether women with PCOS have an increased  
82 risk for cardiovascular disease (CVD). Taking into consideration that many PCOS traits, like irregular cycles,

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3 83 hyperandrogenism, and body mass index (BMI) difference from non-PCOS controls, seem to diminish with  
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5 84 age,[15] it is important to assess CVD-related traits, like cardiac autonomic function, in women with PCOS  
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7 85 also in late reproductive years and beyond menopause to elucidate their possible risk for CVD outcomes.  
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9 86 Previous studies on cardiac autonomic function have included women with PCOS in their 20s or 30s, but to  
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11 87 date no studies have been carried out on women in their late reproductive years. Moreover, the populations  
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13 88 studied have been derived from PCOS clinics; thus, community-based studies are needed. Therefore, the main  
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15 89 aim of this study was to investigate whether women with PCOS from a large, unique general population display  
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17 90 reduced HRV as an indicator of impaired cardiac autonomic function during late reproductive years (at age  
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19 91 46). Additionally, we investigated the role of confounding metabolic abnormalities, such as excess weight,  
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21 92 abdominal obesity, hyperandrogenism, increased blood pressure (BP), dyslipidemia, and insulin resistance, in  
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23 93 cardiac autonomic function in affected women.  
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## 28 95 **MATERIALS AND METHODS**

### 30 96 **Study population**

31 97 The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large,  
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33 98 prospective, general population-based, longitudinal birth cohort. All individuals with expected birth during  
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35 99 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort  
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37 100 (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began  
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39 101 at the 24<sup>th</sup> gestational week, and the women were followed through age 46. The follow-up protocol of the  
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41 102 cohort was previously described in detail, and the main data collection points during adulthood were carried  
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43 103 out at ages 31 and 46 years.[16] Briefly, postal questionnaires were sent to all living cohort members with  
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45 104 known addresses at ages 31 (81% answered,  $n=4,523/5,608$ ) and 46 (72% answered,  $n=3,706/5,123$ ) to collect  
46  
47 105 information about health, behaviour, and social background. Postal questionnaires included an invitation to  
48  
49 106 participate in clinical examinations at age 31 (77% participation rate,  $n=3,127/4,074$ ), and at age 46 (64%  
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51 107 participation rate,  $n=3,280/5,123$ ). Weight and height were self-reported at age 14 (with the help of  
52  
53 108 participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio  
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55 109 of weight (kg) and height squared ( $m^2$ ). Besides anthropometric measurements, clinical examinations at age  
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3 110 46 included blood sampling and assessment of cardiovascular health status, including measurement of systolic  
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5 111 and diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound measurements, and  
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7 112 evaluation of HRV and baroreflex sensitivity. Waist circumference was measured at the level midway between  
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9 113 the lowest rib margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval  
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11 114 after 15 minutes rest, and SBP and DBP averages were calculated.[17] The level of glucose metabolism was  
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13 115 classified according to World Health Organization standards,[18] based on a 2-hour oral glucose tolerance test  
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16 116 (performed at age 46) and a previously established diagnosis of type 2 diabetes.[19]

### 17 18 117 **Definition of PCOS and control groups**

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20 118 At age 31, PCOS symptoms i.e. oligoamenorrhea and hirsutism were self-reported. Of all women who  
21  
22 119 responded to questions regarding PCOS symptoms ( $n=4,523$ ), after excluding pregnant women and those using  
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24 120 hormonal preparations ( $n=1,459$ ) or not permitting the use of their data ( $n=41$ ), 4.1% ( $n=125$ ) reported both  
25  
26 121 oligoamenorrhea and hirsutism. The validity of this questionnaire to distinguish PCOS cases with typical  
27  
28 122 hormonal, metabolic, and psychological traits characteristic to the syndrome, as well as ovarian morphology  
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30 123 for PCOS, has been published previously.[19-22] At age 46, the postal questionnaire included a question on  
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32 124 existing PCOS diagnosis, to which 181 subjects responded “yes.” Consequently, women reporting both  
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34 125 oligoamenorrhea and hirsutism and/or reporting PCOS diagnosis by age 46 were considered cases ( $n=279$ ).  
35  
36 126 Women without PCOS symptoms at age 31 and without diagnosis of PCOS by age 46 were considered controls  
37  
38  
39 127 ( $n=1,577$ ). The characteristics of PCOS and control populations and the flow chart of the formation of the  
40  
41 128 PCOS and control groups have been described previously.[16]

### 42 43 129 **Evaluation of cardiac autonomic function**

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45 130 A flow chart of the study is presented in Figure 1. Heart rate variability was measured in study subjects at age  
46  
47 131 46 in the research unit at Oulu University Hospital and in two other major hospitals nearby. The subjects were  
48  
49 132 informed about the measurement protocol, and an HR monitor (RS800CX, Polar Electro Oy, Kempele,  
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51 133 Finland) to record R-R intervals (RRi) and a standard lead-II ECG (Cardiolife, Nihon Kohden, Tokyo, Japan)  
52  
53 134 were placed on the subjects while seated. Also, breathing frequency (MLT415/D, Nasal Temperature Probe,  
54  
55 135 ADInstruments, Bella Vista, New South Wales, Australia) and BP by finger photoplethysmography (Nexfin,  
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57 136 BMEYE Medical Systems, Amsterdam, the Netherlands) were recorded with a sampling frequency of 1,000  
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59 137 Hz (PowerLab 8/35, ADInstruments). These preparations were followed by at least a 1-minute stabilization



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3 138 period before the beginning of the 3-minute recording period in the seated position. After that recording period,

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5 139 the participants stood up and remained still in a standing position for another three minutes while breathing

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7 140 normally.

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### 9 141 **Analysis of heart rate variability**

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11 142 The first 150 seconds of recording in a seated position and the last 150 seconds in a standing position were

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13 143 used in the analyses. The RRi data were edited based on visual inspection and the artefacts, and ectopic beats

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15 144 were removed and replaced by the local average (Hearts 1.2, University of Oulu, Oulu, Finland). Sequences

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17 145 with  $\geq 10$  consecutive beats of noise or ectopic beats were deleted. The RRi series with  $\geq 80\%$  accepted data

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19 146 were included in the analyses. The final study population included 1,029 controls and 160 women with PCOS.

20

21 147 Mean heart rate, rMSSD (the square root of the mean squared differences of successive normal-to-normal RRi,

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23 148 spectral power densities (fast Fourier transformation, length 512 beats) including low frequency (LF: 0.04 –

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25 149 0.15 Hz,  $\text{ms}^2$ ) and high frequency (HF: 0.15 – 0.40 Hz,  $\text{ms}^2$ ) components of HRV and their ratio (LF/HF) were

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27 150 analysed.

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### 29 151 **Analysis of baroreflex sensitivity**

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31 152 Baroreflex sensitivity was assessed in participants who had the measures performed at the Oulu University

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33 153 Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals were

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35 154 imported to a custom-made stand-alone Matlab-based software (Biosignal Processing Team, University of

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37 155 Oulu, Oulu, Finland), where RRi and SBP values were extracted. Artefacts and ectopic beats were replaced

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39 156 using linear interpolation ( $< 5\%$  for accepted recording) and, thereafter, resampled at 2 Hz and detrended ( $< 0.04$

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41 157 Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128 samples with 50%

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43 158 overlap) was performed to analyse low frequency (LF: 0.04-0.15 Hz) power of RRi and SBP oscillations for

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45 159 subsequent analysis of baroreflex sensitivity by the alpha method, if sufficient coherence ( $\geq 0.5$ ) between LF

46

47 160 oscillations in RRi and SBP was verified.

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### 49 161 **Laboratory methods**

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51 162 The laboratory methods have been previously described in detail.[16] At age 46, sex hormone binding globulin

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53 163 (SHBG) was assayed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Llanberis,

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55 164 UK). The serum samples for testosterone (T) were assayed using Agilent triple quadrupole 6410 LC/MS

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57 165 equipment (Agilent Technologies, Wilmington, DE, USA). Free androgen index (FAI) was calculated using

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3 166 the following equation:  $(100 \times T) / \text{SHBG}$ . Serum total cholesterol, high-density lipoprotein cholesterol (HDL),  
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5 167 low-density lipoprotein cholesterol (LDL), and triglycerides were determined using an enzymatic assay  
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7 168 method. Fasting plasma glucose (f-gluc) was analysed by an enzymatic dehydrogenase method (methods of  
8  
9 169 cholesterol, HDL, LDL, triglycerides, and f-gluc: Advia 1800, Siemens Healthcare Diagnostics Inc.,  
10  
11 170 Tarrytown, NY, USA). Fasting serum insulin (f-ins) was analysed by a chemiluminometric immunoassay  
12  
13 171 (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). High sensitivity C-reactive  
14  
15 172 protein (hsCRP) was analysed by an immune-nephelometric assay (BN ProSpec, Siemens Healthcare  
16  
17 173 Diagnostics Inc., Newark, DE, USA). The f-gluc and f-ins values were used to calculate the Homeostasis  
18  
19 174 Model Assessment–insulin resistance (HOMA–IR) index  $(f\text{-gluc} \times f\text{-ins} / 22.5)$ . The samples were analysed in  
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21 175 NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO  
22  
23 176 15189).

### 26 177 **Hopkins Symptom Check List-25**

28 178 Hopkins Symptom Check List-25, a well-known and widely used symptom inventory, was used in the  
29  
30 179 screening for anxiety. Part 1 includes 10 items that check for anxiety symptoms, and this part was used in  
31  
32 180 the present study. The scale ranges from 1 (not bothersome) to 4 (extremely bothersome).[23]

### 35 181 **Statistical methods**

37 182 Women using beta-blockers (104 controls [7.7%] and 30 women with PCOS [13.3%]) were excluded from the  
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39 183 HRV analysis. Continuous data were presented as mean with standard deviation or as median with 25% and  
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41 184 75% quartiles. Continuous variables with skewed distributions were transformed into a natural logarithm (ln).  
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43 185 Differences in normally distributed continuous parameters were analysed by Student's *t*-test, whereas the  
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45 186 Mann-Whitney *U*-test was used in case of skewed distribution. Categorical data were reported as prevalence  
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47 187 with the number of cases, and the difference between study groups was analysed by cross-tabulation and Chi-  
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49 188 square test or Fisher's exact test, when appropriate. Mean arterial pressure (MAP) was calculated as follows:

$$52 \text{ 189 } \text{DBP} + \frac{1}{3}(\text{SBP} - \text{DBP}).$$

54 190 Univariate and multivariate linear regression analyses were used to study factors associated  
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56 191 with HRV parameters. First, univariate linear regression models were used to reveal the parameters  
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58 192 significantly associated with the outcome variable. Then, stepwise multivariate models were used to identify  
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3 193 the most important explanatory variables. The final multivariate model included the following variables as  
4  
5 194 explanatory variables: PCOS, BMI at age 46, MAP, FAI, HOMA-IR, and triglycerides. The number of  
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7 195 explanatory variables included in the final model had to be limited to avoid multicollinearity. Body mass index  
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9 196 was included in the model, as it significantly differs between PCOS and control women, and obesity is  
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11 197 suggested to affect HRV.[4] Mean arterial pressure was selected because it combines information from both  
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13 198 SBP and DBP, and FAI was included in the model because it is considered a good indicator of  
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15 199 hyperandrogenemia in women with PCOS,[2] and hyperandrogenemia has been suggested to alter HRV in  
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17 200 women with PCOS.[24] Homeostasis Model Assessment-insulin resistance was used as an estimate of insulin  
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19 201 resistance, as it combines information from both fasting insulin and glucose levels, and triglycerides was  
20  
21 202 included, as hypertriglyceridemia is a typical lipid abnormality in PCOS women and is linked to cardiovascular  
22  
23 203 disease risks.[25] Anxiety was not included in the final multivariate model, because in the preliminary models  
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25 204 using the stepwise method, it was always the first variable to be excluded. The results of linear regression  
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27 205 models are reported as unstandardized coefficients (B), 95% confidence interval for B, *P*-value, and  $R^2$  value  
28  
29 206 for the model. The multicollinearity assumptions of the multivariate linear regression model were investigated  
30  
31 207 using VIF, tolerance, and Eigenvalue indexes. In addition, a histogram of regression standardized residual  
32  
33 208 frequency, normal P-P plot of regression-standardized residuals, and scatterplot figures were visually inspected  
34  
35 209 to ensure that the model met the assumptions in the analysis. The data were analysed using SPSS software  
36  
37 210 (IBM SPSS Statistics 24.0, IBM Corp., New York, USA). A *P*-value <0.05 was considered statistically  
38  
39 211 significant.  
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41 212

### 42 212 **Ethical approval**

43 213 The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern  
44  
45 214 Ostrobothnia Hospital District approved the research. All participants took part on a voluntary basis and signed  
46  
47 215 an informed consent.  
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49

### 50 216 **Patient and Public Involvement statement**

51 217 Patients, the public or any third parties were not involved in the design, or conduct, or reporting, or  
52  
53 218 dissemination of our research.  
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## 58 219 **RESULTS**

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### 221 **Heart rate variability and baroreflex sensitivity**

222 When compared with control women, women with PCOS in a seated position had a significantly higher mean  
223 HR and significantly lower values of rMSSD,  $LF_{RRi}$ ,  $HF_{RRi}$ , and baroreflex sensitivity (Figure 2). However,  
224 when adjusting for BMI at age 46, women with PCOS had lower values only in rMSSD ( $P=0.033$ ) and  $HF_{RRi}$   
225 ( $P=0.016$ ) compared with controls. In the standing position, women with PCOS did not differ from controls  
226 after adjustment for BMI at age 46 (data not shown).

### 227 **Heart rate variability according to BMI group**

228 After dividing the women according to BMI ( $<25$  or  $\geq 25$  kg/m<sup>2</sup>), normal weight women with PCOS did not  
229 differ from normal weight control women regarding HRV parameters, whereas overweight/obese women with  
230 PCOS had higher HRs and LF/HF ratios and lower rMSSD,  $LF_{RRi}$ , and  $HF_{RRi}$  compared to overweight/obese  
231 controls (Figure 3). It is noteworthy that overweight/obese women with PCOS had higher BMIs and waist  
232 circumferences and had abnormal glucose metabolism and hyperandrogenemia more often than  
233 overweight/obese control women (data not shown).

### 234 **Linear regression analysis for rMSSD**

235 The univariate linear regression analysis demonstrated that rMSSD was associated with BMI at ages 31 and  
236 46 and with waist circumference, SBP, DBP, MAP, serum levels of total cholesterol, HDL, LDL, triglycerides,  
237 fasting glucose, fasting insulin, HOMA-IR, SHBG, FAI, hsCRP, and anxiety at age 46 (Table 1). Body mass  
238 index at age 14 was not associated with rMSSD. The multivariate linear regression analysis demonstrated that  
239 MAP, HOMA-IR, and triglycerides were the strongest explanatory variables for rMSSD (Table 1).

**Table 1.** Univariate and multivariate linear regression models for heart rate variability measure (rMSSD) in women with PCOS.

Variable	Univariate regression analysis				Multivariate Model* ( $B=4.075$ , $R^2=0.101$ )		
	B	95%CI for B	P-value	R <sup>2</sup>	B	95%CI for B	P-value
PCOS	NA	NA	NA	NA	-0.087	-0.190 – 0.016	0.099
BMI 14yr	-0.011	-0.022 – 0.000	0.057	0.002			
BMI 31yr	-0.022	-0.028 – -0.016	<0.001	0.022			
BMI 46yr	-0.028	-0.033 – -0.023	<0.001	0.055	-0.007	-0.016 – 0.002	0.140
Waist circumference	-0.012	-0.014 – -0.010	<0.001	0.061			
Systolic BP	-0.007	-0.009 – -0.005	<0.001	0.032			
Diastolic BP	-0.015	-0.017 – -0.013	<0.001	0.066			
Mean arterial pressure	-0.012	-0.014 – -0.010	<0.001	0.054	-0.007	-0.011 – -0.004	<0.001
Testosterone	0.042	-0.010 – 0.093	0.113	0.001			
SHBG	0.001	0.001 – 0.002	<0.001	0.005			
FAI	-0.121	-0.199 – -0.042	0.003	0.003	0.059	-0.055 – 0.173	0.309
Glucose	-0.121	-0.153 – -0.089	<0.001	0.023			
Insulin	-0.014	-0.018 – -0.011	<0.001	0.027			
HOMA-IR	-0.492	-0.585 – -0.399	<0.001	0.049	-0.256	-0.420 – -0.092	0.002
Total cholesterol	-0.067	-0.096 – -0.038	<0.001	0.009			
High density lipoprotein	0.138	0.076 – 0.201	<0.001	0.008			
Low density lipoprotein	-0.086	-0.114 – -0.058	<0.001	0.015			
Triglycerides	-0.243	-0.285 – -0.200	<0.001	0.050	-0.341	-0.558 – -0.123	0.002
High sensitive CRP	-0.071	-0.084 – -0.057	<0.001	0.034			
Anxiety (HSCL-25)	-0.195	-0.274 – -0.117	<0.001	0.010			

NA: not applicable. BMI: body mass index, BP: blood pressure, SHBG: sex hormone binding globulin, FAI: free androgen index, HOMA-IR: Homeostasis Model Assessment–insulin resistance, CRP: C-reactive protein.

\* The multivariate model included PCOS, BMI, mean arterial pressure, FAI, HOMA-IR, and triglycerides as explanatory variables. B: unstandardized coefficient from linear regression analysis, 95% CI: 95% confidence interval. FAI and HOMA-IR were ln-transformed to achieve normal distribution.

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3 248 **Hyperandrogenemia**  
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5 249 Total or calculated free T at age 46 did not associate with rMSSD in linear regression analysis (data not shown).  
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7 250 Free androgen index was negatively associated with rMSSD (B= -0.121, 95% CI: -0.199 – -0.042, *P*=0.003),  
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9 251 but lost its significance after adjustment for BMI. Similarly, serum level of SHBG was positively associated  
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11 252 with rMSSD (B=0.001, 95% CI: 0.001 – 0.002, *P*<0.001), but lost its significance after BMI adjustment.  
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## 253 DISCUSSION

254 To our knowledge, this is the largest general population-based study on women of late reproductive age with  
255 PCOS investigating cardiac autonomic function to date. Here, we demonstrate that women with PCOS display  
256 reduced vagal activity at age 46. The alterations were associated with metabolic abnormalities, such as elevated  
257 BP, insulin resistance, and dyslipidemia. We did not find an association between hyperandrogenemia and HRV  
258 parameters after adjustment for BMI.

259 Previous studies have reported that women with PCOS display impaired cardiac autonomic  
260 function with increased sympathetic activity and decreased parasympathetic and overall cardiac  
261 modulation.[10-12] In line with our findings, a study of 75 overweight women with PCOS and 75 age- and  
262 BMI-matched controls suggested that impaired HR recovery, a marker for decreased parasympathetic activity,  
263 was caused by excess weight and insulin resistance and not by PCOS *per se*. [26] Furthermore, a cross-sectional  
264 study of 31 PCOS cases recruited from outpatient clinics reported that women with PCOS showed significantly  
265 decreased vagal activity, but the role of confounding metabolic abnormalities was not assessed, even though  
266 women with PCOS had significantly higher BMIs, waist-hip ratios, BP, T, and fasting glucose levels than  
267 control women.[10] In line with these studies, we also found that women with PCOS displayed mainly reduced  
268 vagal activity at age 46.

269 In the present study overweight/obese women with PCOS had more adverse changes in HRV  
270 parameters than overweight/obese control women, whereas normal-weight PCOS and control groups had  
271 comparable HRV values. However, the difference between overweight/obese PCOS and control groups may  
272 have reflected the higher mean BMIs and waist circumferences as well as the higher prevalence of abnormal  
273 glucose metabolism in the overweight/obese PCOS group than in the overweight/obese controls. Previous  
274 studies have reported conflicting results regarding the effect of BMI on autonomic function in PCOS. A study  
275 of 19 overweight/obese women with PCOS and 21 overweight/obese control women reported that women with  
276 PCOS had elevated multi-unit and single-unit muscle sympathetic nervous system activity, whereas HRV  
277 parameters did not significantly differ between the groups.[13] On the other hand, it was reported that non-  
278 obese (BMI<30kg/m<sup>2</sup>) control and PCOS groups did not have significantly different standard deviation of all  
279 RRis (SDNN), rMSSD, or percentage of successive differences in RRi > 50 ms (pNN50), whereas obese  
280 (BMI≥30 kg/m<sup>2</sup>) women with PCOS had significantly decreased SDNN and pNN50, but not rMSSD.[27]

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3 281 Findings of that study also demonstrated that sympathetic skin responses, investigated by electromyography  
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5 282 from the median or tibial nerve, were altered in both non-obese and obese PCOS groups. These conflicting  
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7 283 results might be explained by the fact that the effect of obesity in sympathetic activation might be regional, as  
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9 284 obesity has been reported to increase sympathetic activity in the kidneys and skeletal muscle vasculature, but  
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11 285 to reduce it in the heart.[28]

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14 286           Previous results regarding the effect of hyperandrogenemia on the cardiac autonomic function  
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16 287 in PCOS have been conflicting. In our data, serum T or FAI at age 46 did not associate with HRV after BMI  
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18 288 adjustment (nor did FAI or T at age 31, data not shown). In line with our findings, two previous studies found  
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20 289 no significant association between T and LF [12] or hormonal profile and HR recovery.[11] By contrast, total  
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22 290 T level was reported to be inversely associated with LF and LF/HF ratio in women with PCOS during mental  
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24 291 stress testing.[29] Another study found higher muscle sympathetic nerve activity (MSNA) in normal weight  
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26 292 women with PCOS compared with normal weight controls, and the strongest explanatory factors for higher  
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28 293 MSNA in women with PCOS were total and free T and cholesterol.[24] A plausible explanation for these  
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30 294 conflicting findings is that the previous studies used various T determination methods, such as immunoassays  
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32 295 [24, 30] and chemiluminescence,[29] whereas we used the golden standard, liquid-tandem mass-spectrometry  
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34 296 assay for T measurement. However, it is also possible that the severities of both PCOS and hyperandrogenism  
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36 297 may influence the degree of sympatoexcitation,[24] and our study population, with rather low T levels, may,  
37  
38 298 therefore, not present excess sympathetic activity.

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41 299           Also, the phenotype of PCOS has been suggested to influence cardiac autonomic function, as  
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43 300 the anovulatory women with PCOS showed lower HRV response in mental stress tests than controls, whereas  
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45 301 ovulatory PCOS women showed intermediate values.[29] However, in that study women with anovulatory  
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47 302 PCOS had a different metabolic profile than those with ovulatory PCOS and control groups.[29] Furthermore,  
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49 303 previous studies indicated that anxiety was associated with reduced HRV in the general population.[8] In our  
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51 304 analysis, anxiety had a weak association with rMSSD in the univariate linear regression model, although  
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53 305 further analysis revealed that metabolic abnormalities played a more important role in reduction of vagal  
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55 306 activity.

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58 307           In the present study, BP, insulin resistance, and dyslipidemia were associated with rMSSD in  
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60 308 the multivariate linear regression analysis, indicating that metabolic abnormalities are likely to be the main



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cause for reduced HRV in women with PCOS. Our findings are in line with previous studies conducted in younger women in which an association between BP, glucose metabolism, dyslipidemia, and cardiac autonomic function in the general population was reported.[5, 31] A recent study addressed the inter-related effects of insulin resistance, hyperandrogenism, chronic inflammation, and sympathetic dysfunction (evaluated by MSNA) in 49 PCOS and 23 control women; based on the findings it was concluded that sympathetic dysfunction and hyperandrogenism were associated with PCOS, and that chronic inflammation might be the mediating factor between sympathetic function, hyperandrogenism, and insulin resistance.[32] In line with this, we found that hsCRP was negatively associated with rMSSD in the univariate linear regression analysis.

The strength of our study is that it includes by far the largest sample size of women with PCOS and HRV measurement. The data also adds to the literature by representing a community-based approach. Moreover, we were able to adjust for many confounding factors and to study the effect of metabolic abnormalities. Also, this is the first study to investigate women with PCOS at a late fertile age. The definition of the PCOS population could be considered as a limitation; however, we have previously shown that the population does display the typical endocrine, metabolic, and psychological profiles of PCOS.[20-22] Moreover, a recent genome-wide meta-analysis reported that the genetic architecture does not differ based on the diagnostic criteria used for PCOS (self-reported, NIH criteria, or non-NIH Rotterdam criteria),[33] thus supporting our approach. Our study population included only women with Caucasian ethnicity; consequently, our results are best generalized to PCOS women with Caucasian ethnicity, as ethnicity is known to affect many traits of PCOS.

In conclusion, in this community-based data set, women with PCOS displayed an impaired cardiac autonomic function at a late fertile age with strong association with metabolic abnormalities. The fact that impaired cardiovascular autonomic function, i.e., increased sympathetic/decreased parasympathetic activity, reflects the risk for cardiac morbidity; this is most likely also the case in PCOS. This underlines the importance of active screening and treatment of metabolic abnormalities in women with PCOS, as also suggested by the recently published guidelines for PCOS.[34] Previous studies have reported that in overweight/obese women with PCOS, the impaired cardiovascular autonomic function could be improved by a 10-week energy restriction,[30] a 3-month aerobic exercise training program,[35] or acupuncture.[36]

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3 336 Importantly, it has been shown that by weight reduction, the autonomic disturbance can be reversed,[37] setting  
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5 337 the goal for increasing resources and efforts targeting weight management in affected women.  
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11 340 **A DATA STATEMENT:** Data are available on request to the NFBC1966 Data Sharing Committee.  
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13 341 NFBC1966 data sharing policies and processes meet the requirement and expectations of Northern  
14  
15 342 Ostrobothnia Hospital district policy on sharing of data from population and patient cohorts. Further  
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17 343 information can be found at <https://www oulu fi/nfbc/>

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20 344 **DECLARATION OF INTEREST:** None declared.  
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47 357 **AUTHOR CONTRIBUTIONS:** M-M.O.: study design, data analyses and interpretation, manuscript drafting  
48  
49 358 and revision, A.K.: study design, data collection, analyses, and interpretation, manuscript drafting and revision,  
50  
51 359 E.S-V.: data interpretation, manuscript drafting, and revision, M.T.: data collection and interpretation,  
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53 360 manuscript revision, K.P.: laboratory analyses, manuscript revision, J.S.T.: data interpretation, manuscript  
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55 361 revision, S.F.: data interpretation, manuscript revision, L.M-P.: data interpretation, manuscript drafting and  
56  
57 362 revision, and T.T.P.: study design, data interpretation, manuscript drafting and revision.  
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60 363 **REFERENCES**

- 1  
2  
3 364 1 March WA, Moore VM, Willson KJ, et al. The prevalence of polycystic ovary syndrome in a community  
4  
5 365 sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;25:544–51.  
6  
7 366 2 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on  
8  
9 367 diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–  
10  
11 368 25.  
12  
13 369 3 Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;333:853–61.  
14  
15 370 4 Lambert GW, Straznicky NE, Lambert EA, et al. Sympathetic nervous activation in obesity and the metabolic  
16  
17 syndrome--causes, consequences and therapeutic implications. *Pharmacol Ther* 2010;126:159–72.  
18 371  
19  
20 372 5 Stuckey MI, Tulppo MP, Kiviniemi AM, et al. Heart rate variability and the metabolic syndrome: a  
21  
22 373 systematic review of the literature. *Diabetes Metab Res Rev* 2014;30:784–93. doi:10.1002/dmrr.2555  
23  
24 374 6 Kakoly NS, Moran LJ, Teede HJ, et al. Cardiometabolic risks in PCOS: a review of the current state of  
25  
26 375 knowledge. *Expert Rev Endocrinol Metab* 2019;14:23–33.  
27  
28 376 7 Sgoifo A, Carnevali L, Alfonso Mde L, et al. Autonomic dysfunction and heart rate variability in depression.  
29  
30 377 *Stress* 2015;18:343–52. doi:10.3109/10253890.2015.1045868.  
31  
32 378 8 Paniccia M, Paniccia D, Thomas S, et al. Clinical and non-clinical depression and anxiety in young people:  
33  
34 A scoping review on heart rate variability. *Auton Neurosci* 2017;208:1–14. doi:10.1016/j.autneu.2017.08.008  
35 379  
36  
37 380 9 Wulsin LR, Horn PS, Perry JL, et al. Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular  
38  
39 381 Disease, Diabetes, and Mortality. *J Clin Endocrinol Metab* 2015;100:2443–8. doi:10.1210/jc.2015-1748  
40  
41 382 10 Saranya K, Pal GK, Habeebullah S, et al. Assessment of cardiovascular autonomic function in patients with  
42  
43 383 polycystic ovary syndrome. *J Obstet Gynaecol Res* 2014;40:192–9. doi:10.1111/jog.12154  
44  
45 384 11 Tekin G, Tekin A, Kiliçarslan EB, et al. Altered autonomic neural control of the cardiovascular system in  
46  
47 385 patients with polycystic ovary syndrome. *Int J Cardiol* 2008;130:49–55. doi:10.1016/j.ijcard.2007.08.037  
48  
49 386 12 Yildirim A, Aybar F, Kabakci G, et al. Heart rate variability in young women with polycystic ovary  
50  
51 387 syndrome. *Ann Noninvasive Electrocardiol* 2006;11:306–12. doi:10.1111/j.1542-474X.2006.00122.x  
52  
53 388 13 Lambert EA, Teede H, Sari CI, et al. Sympathetic activation and endothelial dysfunction in polycystic ovary  
54  
55 389 syndrome are not explained by either obesity or insulin resistance. *Clin Endocrinol (Oxf)* 2015;83:812–9.  
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57 doi:10.1111/cen.12803  
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57  
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60

- 391 14 Anonymous. Heart rate variability: standards of measurement, physiological interpretation and clinical use.  
392 Task Force of the European Society of Cardiology and the North American Society of Pacing and  
393 Electrophysiology. *Circulation* 1996;93:1043–65.
- 394 15 Brown ZA, Louwers YV, Fong SL, et al. The phenotype of polycystic ovary syndrome ameliorates with  
395 aging. *Fertil Steril* 2011;96:1259–65. doi:10.1016/j.fertnstert.2011.09.002
- 396 16 Ollila MM, Piltonen T, Puukka K, et al. Weight Gain and Dyslipidemia in Early Adulthood Associate With  
397 Polycystic Ovary Syndrome: Prospective Cohort Study. *J Clin Endocrinol Metab* 2016;101:739–47.  
398 doi://dx.doi.org/10.1210/jc.2015-3543
- 399 17 Ollila ME, Kaikkonen K, Järvelin M, et al. Self-Reported Polycystic Ovary Syndrome Is Associated With  
400 Hypertension: A Northern Finland Birth Cohort 1966 Study. *J Clin Endocrinol Metab* 2019;104:1221–31.  
401 doi:10.1210/jc.2018-00570
- 402 18 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications.  
403 Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*  
404 1998;15:539–53.
- 405 19 Ollila MM, West S, Keinänen-Kiukaanniemi S, et al. Overweight and obese but not normal weight women  
406 with PCOS are at increased risk of Type 2 diabetes mellitus—a prospective, population-based cohort study. *Hum*  
407 *Reprod* 2017;32:423–31. doi: 10.1093/humrep/dew329
- 408 20 Karjula S, Morin-Papunen L, Auvinen J, et al. Psychological Distress Is More Prevalent in Fertile Age and  
409 Premenopausal Women With PCOS Symptoms: 15-Year Follow-Up. *J Clin Endocrinol Metab*  
410 2017;102:1861–9. doi://dx.doi.org/10.1210/jc.2016-3863
- 411 21 Taponen S, Martikainen H, Järvelin MR, et al. Hormonal profile of women with self-reported symptoms of  
412 oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab*  
413 2003;88:141–7.
- 414 22 Taponen S, Ahonkallio S, Martikainen H, et al. Prevalence of polycystic ovaries in women with self-  
415 reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *Hum*  
416 *Reprod* 2004;19:1083–8.
- 417 23 Veijola J, Jokelainen J, Läksy K, et al. The Hopkins Symptom Checklist-25 in screening DSM-III-R axis-  
418 I disorders. *Nord J Psychiatry* 2003;57:119–23. doi:10.1080/08039480310000941.

- 1  
2  
3 419 24 Sverrisdóttir YB, Mogren T, Kataoka J, et al. Is polycystic ovary syndrome associated with high  
4  
5 420 sympathetic nerve activity and size at birth? *Am J Physiol Endocrinol Metab* 2008;294:576–81.  
6  
7 421 doi:10.1152/ajpendo.00725.2007  
8  
9 422 25 Fauser BC, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary  
10  
11 423 syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil*  
12  
13 424 *Steril* 2012;97:28–38.e25. doi: 10.1016/j.fertnstert.2011.09.024  
14  
15  
16 425 26 Giallauria F, Palomba S, Manguso F, et al. Abnormal heart rate recovery after maximal cardiopulmonary  
17  
18 426 exercise stress testing in young overweight women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
19  
20 427 2008;68:88–93. doi:10.1111/j.1365-2265.2007.03004.x  
21  
22 428 27 Hashim ZH, Hamdan FB, Al-Salihi AR. Autonomic dysfunction in women with polycystic ovary syndrome.  
23  
24 429 *Iran J Reprod Med* 2015;13:27–34.  
25  
26 430 28 Lansdown A, Rees DA. The sympathetic nervous system in polycystic ovary syndrome: a novel therapeutic  
27  
28 431 target? *Clin Endocrinol (Oxf)* 2012;77:791–801. doi:10.1111/cen.12003  
29  
30 432 29 Di Domenico K, Wiltgen D, Nickel FJ, et al. Cardiac autonomic modulation in polycystic ovary syndrome:  
31  
32 433 does the phenotype matter? *Fertil Steril* 2013;99:286–92. doi:10.1016/j.fertnstert.2012.08.049  
33  
34  
35 434 30 Thomson RL, Buckley JD, Noakes M, et al. Heart rate recovery improves after weight loss in overweight  
36  
37 435 and obese women with polycystic ovary syndrome. *Fertil Steril* 2010;93:1173–8  
38  
39 436 doi:10.1016/j.fertnstert.2008.12.003  
40  
41 437 31 Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability  
42  
43 438 and cardiovascular disease risk factors. *Int J Cardiol* 2010;141:122–31. doi:10.1016/j.ijcard.2009.09.543  
44  
45 439 32 Shorakae S, Ranasinha S, Abell S, et al. Inter-related effects of insulin resistance, hyperandrogenism,  
46  
47 440 sympathetic dysfunction and chronic inflammation in PCOS. *Clin Endocrinol (Oxf)* 2018;89:628–33.  
48  
49 441 doi:10.1111/cen.13808  
50  
51 442 33 Day F, Karaderi T, Jones MR, et al. Large-scale genome-wide meta-analysis of polycystic ovary syndrome  
52  
53 443 suggests shared genetic architecture for different diagnosis criteria. *PLoS Genet* 2018;14:e1007813  
54  
55 444 doi:10.1371/journal.pgen.1007813  
56  
57  
58  
59  
60

3 445 34 Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based  
4  
5 446 guideline for the assessment and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
6  
7 447 2018;89:251–268. doi:10.1111/cen.13795.

9  
10 448 35 Giallauria F, Palomba S, Maresca L, et al. Exercise training improves autonomic function and inflammatory  
11  
12 449 pattern in women with polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2008;69:792–8.  
13  
14 450 doi:10.1111/j.1365-2265.2008.03305.x

15  
16 451 36 Stener-Victorin E, Jedel E, Janson PO, et al. Low-frequency electroacupuncture and physical exercise  
17  
18 452 decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am J Physiol Regul Integr*  
19  
20 453 *Comp Physiol* 2009;297:387–95. doi:10.1152/ajpregu.00197.2009

21  
22 454 37 Maser RE, Lenhard MJ. An overview of the effect of weight loss on cardiovascular autonomic function.  
23  
24 455 *Curr Diabetes Rev* 2007;3:204–11.

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## 28 457 **FIGURE LEGENDS**

30 458 **Figure 1.** Flow chart of the study. RRi: R-R interval.

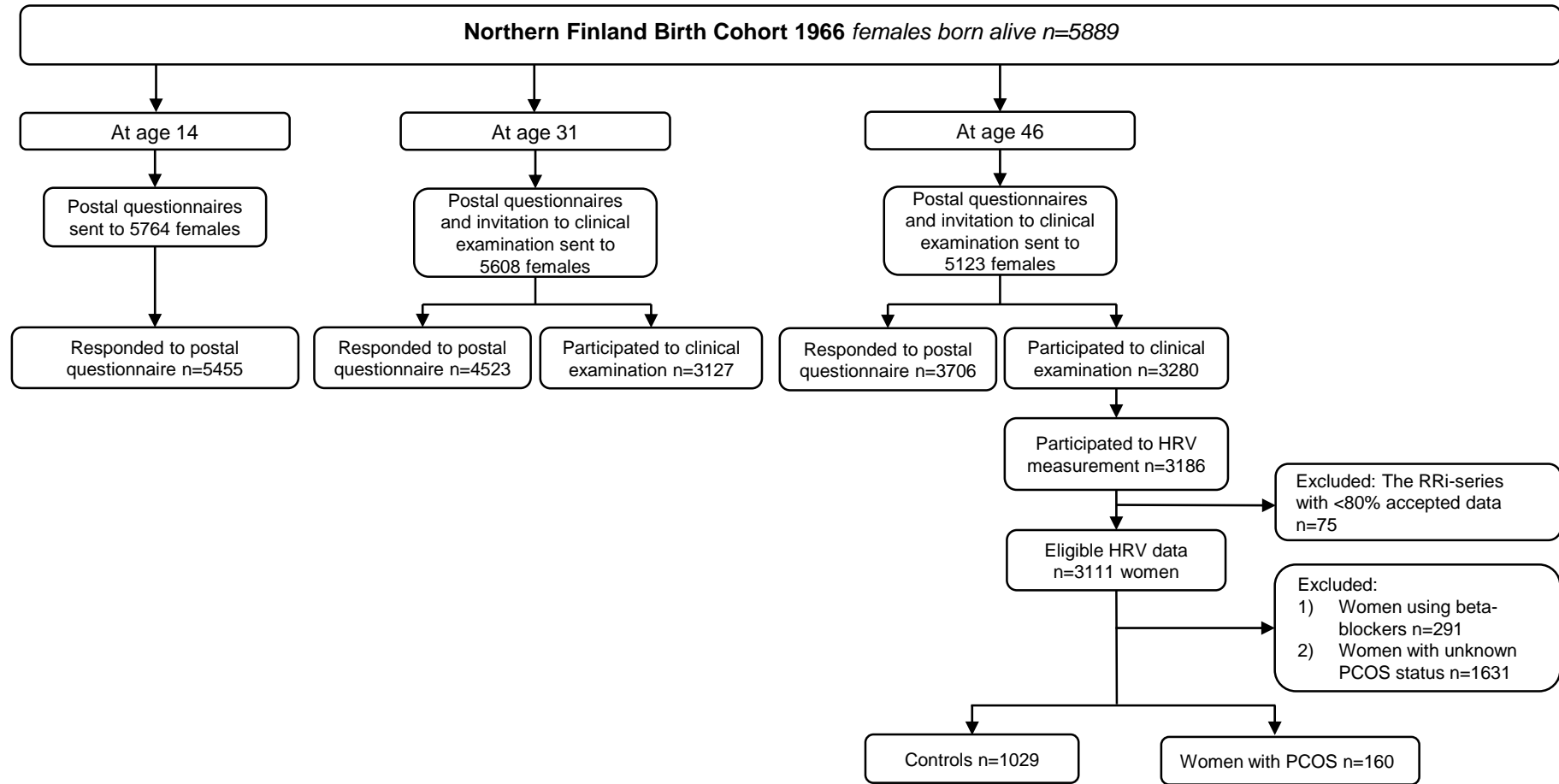
32 459 **Figure 2.** Heart rate variability parameters in controls and in women with PCOS at age 46 in seated position.  
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35 460 The users of beta-blockers were excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles and  
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37 461 the significance testing was made by Student's *t*-test (ln-transform was made to achieve normality). HR: heart  
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39 462 rate. rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals  
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41 463 (RRi), LF<sub>RRi</sub>: low frequency (0.04-0.15 Hz) power, HF<sub>RRi</sub>: high frequency (0.15-0.4 Hz) power,  $\alpha_1$ : short-term  
42  
43 464 fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex  
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45 465 sensitivity.

47 466 **Figure 3.** Heart rate variability parameters in controls and in women with PCOS at age 46 according to the  
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49 467 BMI group. The assessment of autonomic function in seated position. The users of beta-blockers were  
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51 468 excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles and the significance testing was made  
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53 469 by Student's *t*-test (ln-transform was made to achieve normality). Statistically significant *P*-values are bolded.  
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55 470 rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals (RRi),  
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57 471 LF<sub>RRi</sub>: low frequency (0.04-0.15 Hz) power, HF<sub>RRi</sub>: high frequency (0.15-0.4 Hz) power,  $\alpha_1$ : short-term fractal-

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472 like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex  
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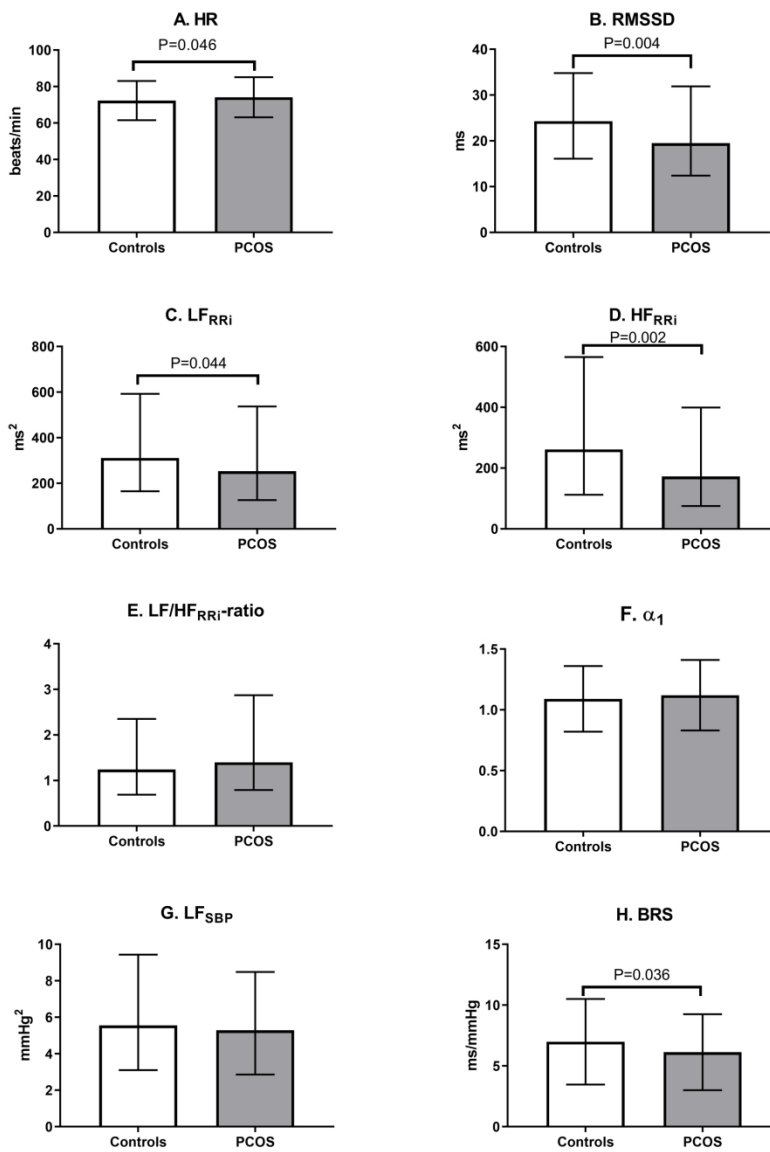


Figure 2

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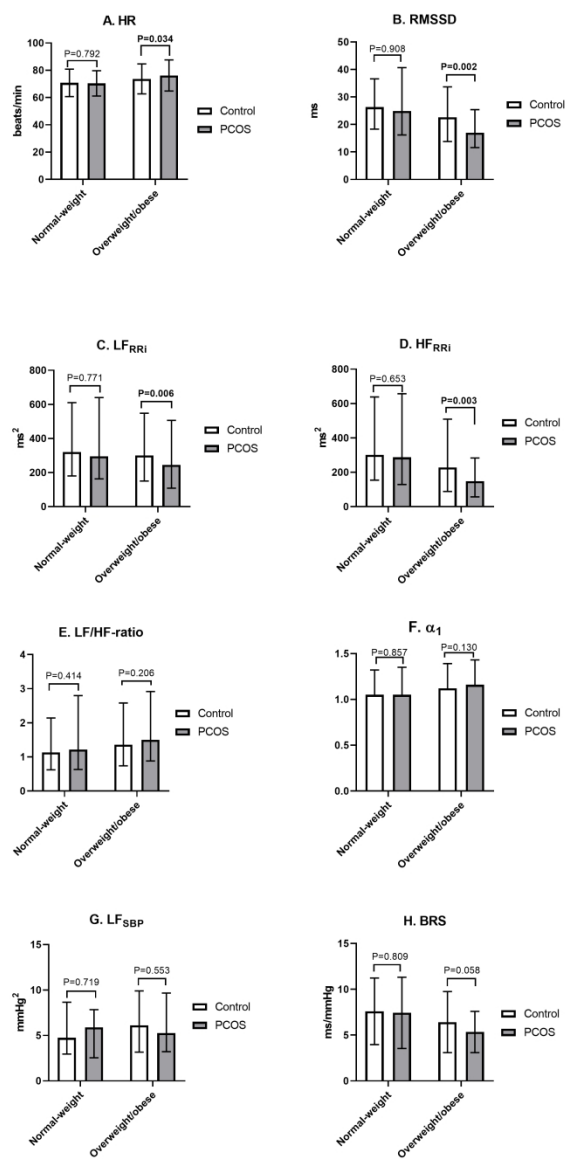


Figure 3

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STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	Page 1,2	
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4	
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4	
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	Page 5 and 6	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5 and 6	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	Page 5 and 6	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pages 6 – 8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 6 – 8	
Bias	9	Describe any efforts to address potential sources of bias	Page 15	
Study size	10	Explain how the study size was arrived at	Page 5 and 6	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 8 and 9
		(b) Describe any methods used to examine subgroups and interactions	Page 8 and 9
		(c) Explain how missing data were addressed	NA
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	NA
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pages 5 – 7
		(b) Give reasons for non-participation at each stage	Pages 5 – 7
		(c) Consider use of a flow diagram	Flow chart is included
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	Page 5
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 9 – 12
		(b) Report category boundaries when continuous variables were categorized	Pages 9 – 10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Pages 13–15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 13–15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 15
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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

# BMJ Open

## THE EFFECT OF POLYCYSTIC OVARY SYNDROME ON CARDIAC AUTONOMIC FUNCTION AT A LATE FERTILE AGE: A PROSPECTIVE NORTHERN FINLAND 1966 BIRTH COHORT STUDY

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<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Cardiovascular medicine, Reproductive medicine
Keywords:	PCOS, Heart rate, Heart rate variability, Obesity, Metabolism

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Manuscripts

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9 3 **BIRTH COHORT STUDY**  
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13 5 Meri-Maija Ollila<sup>1</sup>, Antti M. Kiviniemi<sup>2</sup>, Elisabet Stener-Victorin<sup>3</sup>, Mikko Tulppo<sup>2</sup>, Katri Puukka<sup>4</sup>, Juha S.  
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**ABSTRACT**

**Objectives:** Previous studies of women in their 20s and 30s have reported impaired autonomic function in women with polycystic ovary syndrome (PCOS). We aimed to study, for the first time, whether PCOS is associated with impaired cardiac autonomic function independent of metabolic and hormonal status in their late reproductive years.

**Design:** A prospective Northern Finland birth cohort (NFBC66) study including 5,889 females born in 1966 and followed through the age of 46. At that age,  $n=3,706/5,123$  women (72%) answered the postal questionnaires and  $n=3,280/5,123$  women (64%) participated to the clinical examination.

**Setting:** General community.

**Participants:** The sample included women presenting both irregular menses (oligomenorrhea or amenorrhea) and hirsutism at age 31 ( $n=125$ ) or with formally diagnosed PCOS by age 46 ( $n=181$ ) and women without PCOS symptoms or diagnosis ( $n=1,577$ ).

**Primary and secondary outcome measures:** Heart rate variability parameters: the root mean square of successive R–R differences (rMSSD), spectral power densities (LF: low frequency and HF: high frequency), and baroreflex sensitivity (BRS).

**Results:** We found that parasympathetic activity (assessed by rMSSD: 19.5 [12.4; 31.9] vs. 24.3 [16.1; 34.8] ms,  $P=0.004$  and HF: 172 [75; 399] vs. 261 [112; 565] ms<sup>2</sup>,  $P=0.002$ ) and BRS ( $6.13\pm 3.12$  vs.  $6.99\pm 3.52$  ms/mmHg,  $P=0.036$ ) were lower in women with PCOS compared with the controls.

However, in the multivariate regression analysis, PCOS, body mass index and the free androgen index did not significantly associate with rMSSD, whereas blood pressure, insulin resistance and triglycerides did.

**Conclusions:** We report here for the first time that late reproductive-aged women with PCOS display impaired cardiac autonomic function manifested as decreased vagal activity. Metabolic status, rather



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4 48 than hyperandrogenemia and PCOS *per se*, was the strongest contributing factor. Given the link  
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7 49 between cardiac morbidity and impaired autonomic function, the findings underline the importance  
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10 50 of screening and treating metabolic abnormalities early on in women with PCOS.

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16 52 **Keywords:** PCOS, heart rate, heart rate variability, obesity, metabolism

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For peer review only

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3 53 **Strengths and limitations of this study**  
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- 5 54 • This is the first study to investigate the cardiac autonomic function of late reproductive aged  
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9 55 women.  
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12 56 • This study provides the largest study population by far compared to the previous studies in  
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15 57 cardiac autonomic function in women with PCOS.  
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18 58 • We were able to adjust for many confounding factors and to study the effect of metabolic  
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21 59 abnormalities.  
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24 60 • The study is limited by the lack of PCOS phenotypes.  
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28 61 • The study cannot be generalized with all ethnicities.  
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## 62 INTRODUCTION

63 Polycystic ovary syndrome (PCOS) is the most common endocrinopathy, affecting 6–18% of women  
64 of reproductive age and characterized by irregular menstruation, clinical or biochemical  
65 hyperandrogenism, and polycystic ovaries.[1-3] Women with PCOS are commonly overweight or  
66 obese and typically present with insulin resistance, hyperinsulinemia, increased blood pressure,  
67 dyslipidemia, metabolic syndrome and obstructive sleep apnea,[2] all of which are associated with  
68 impaired cardiac autonomic function.[4-6] In the general population, the dysregulation of cardiac  
69 autonomic function has been associated with increased risk of many major global public health  
70 problems, such as depression,[7] anxiety,[8] hypertension, diabetes, cardiovascular diseases and  
71 mortality.[9] Therefore, it is not surprising that women with PCOS have been shown to present with  
72 impaired cardiac autonomic function; that is, reduced parasympathetic (vagal) activity,[10-12] and  
73 increased sympathetic nervous system activity.[13, 14] Previous researchers have used various  
74 methods to assess the cardiac autonomic function in women with PCOS, such as microneurography,  
75 the measurement of sympathetic skin responses, heart rate (HR) variability (HRV), HR recovery,  
76 and a noradrenaline spill-over measurement. Of these methods, the measurement of HRV (i.e.  
77 variations in the time intervals between consecutive heartbeats), provides a well-established non-  
78 invasive method to assess, in particular, parasympathetic cardiac autonomic activity.[15]

79 During recent years, it has become an enigma whether women with PCOS have an increased  
80 risk for cardiovascular disease (CVD). Taking into consideration that many PCOS traits, such as irregular

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3 81 cycles, hyperandrogenism and body mass index (BMI) difference from non-PCOS controls, seem to diminish  
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5 82 with age,[16] it is also important to assess CVD-related traits, such as cardiac autonomic function, in women  
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7 83 with PCOS in the late reproductive years and beyond menopause to elucidate their possible risk for CVD  
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9 84 outcomes. Previous studies on cardiac autonomic function have included women with PCOS in their 20s or  
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11 85 30s, but to date, no studies have been carried out on women in their late reproductive years. Moreover, the  
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13 86 populations studied have been derived from PCOS clinics; thus, community-based studies are needed.  
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15 87 Therefore, the main aim of this study was to investigate whether women with PCOS from a general population  
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17 88 display reduced HRV as an indicator of impaired cardiac autonomic function during their late reproductive  
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19 89 years (at age 46). Additionally, we investigated the role of confounding metabolic abnormalities, such as  
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21 90 excess weight, abdominal obesity, hyperandrogenism, increased blood pressure (BP), dyslipidemia and insulin  
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23 91 resistance, in the cardiac autonomic function in affected women.  
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## 28 93 **MATERIALS AND METHODS**

### 30 94 **Study population**

32 95 The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large,  
34 96 prospective, general population-based, longitudinal birth cohort. All individuals with expected births during  
36 97 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort  
38 98 (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began  
40 99 at the 24<sup>th</sup> gestational week, and the women were followed through age 46. The follow-up protocol of the  
42 100 cohort was previously described in detail, and the main data collection points during adulthood were carried  
44 101 out at ages 31 and 46 years.[17] Briefly, postal questionnaires were sent to all living cohort members with  
46 102 known addresses at ages 31 (81% answered,  $n=4,523/5,608$ ) and 46 (72% answered,  $n=3,706/5,123$ ) to collect  
48 103 information about health, behaviour and social background. Postal questionnaires included an invitation to  
50 104 participate in clinical examinations at age 31 (77% participation rate,  $n=3,127/4,074$ ) and at age 46 (64%  
52 105 participation rate,  $n=3,280/5,123$ ). Weight and height were self-reported at age 14 (with the help of the  
54 106 participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio  
56 107 of weight (kg) and height squared ( $m^2$ ). Besides anthropometric measurements, the clinical examinations at  
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3 108 age 46 included blood sampling and assessments of cardiovascular health status, including systolic and  
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5 109 diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound, and evaluations of HRV  
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7 110 and baroreflex sensitivity. Waist circumference was measured at the level midway between the lowest rib  
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9 111 margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval after 15  
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11 112 minutes rest by an automated, oscillometric BP device with an appropriately sized cuff (Omron Digital  
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13 113 Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan), and SBP and DBP averages were  
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16 114 calculated.[18] The level of glucose metabolism was classified according to World Health Organization  
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18 115 standards,[19] based on a 2-hour oral glucose tolerance test (performed at age 46) and a previously established  
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20 116 diagnosis of type 2 diabetes.[20]

### 21 22 117 **Definition of PCOS and control groups**

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24 118 At age 31, PCOS symptoms (i.e. oligomenorrhea/amenorrhea and hirsutism) were self-reported. Of all the  
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26 119 women who responded to questions regarding PCOS symptoms ( $n=4,523$ ), after excluding pregnant women  
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28 120 and those using hormonal preparations ( $n=1,459$ ) or not permitting the use of their data ( $n=41$ ), 4.1% ( $n=125$ )  
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30 121 reported both oligomenorrhea/amenorrhea and hirsutism. The validity of this questionnaire to distinguish  
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32 122 PCOS cases with typical hormonal, metabolic and psychological traits characteristic to the syndrome, as well  
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35 123 as ovarian morphology for PCOS, has previously been described.[20-23] At age 46, the postal questionnaire  
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37 124 included a question on existing PCOS diagnosis, to which 181 subjects responded “yes.” Consequently, the  
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39 125 women reporting both oligomenorrhea/amenorrhea and hirsutism and/or reporting PCOS diagnosis by age 46  
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41 126 were considered cases ( $n=279$ ). Women without PCOS symptoms at age 31 and without diagnosis of PCOS  
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43 127 by age 46 were considered controls ( $n=1,577$ ). The characteristics of the PCOS and control populations and  
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45 128 the flow chart of the formation of the PCOS and control groups have previously been described.[17]

### 46 47 129 **Evaluation of cardiac autonomic function**

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49 130 A flow chart of the study is presented in Figure 1. Heart rate variability was measured in the study subjects at  
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51 131 age 46 in the research unit at Oulu University Hospital and in two other major hospitals nearby. The subjects  
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53 132 were informed about the measurement protocol, and an HR monitor (RS800CX, Polar Electro Oy, Kempele,  
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55 133 Finland) to record R-R intervals (RRi) and a standard lead-II ECG (Cardiolife, Nihon Kohden, Tokyo, Japan)  
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58 134 were placed on the subjects while seated. Also, breathing frequency (MLT415/D, Nasal Temperature Probe,  
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60 135 ADInstruments, Bella Vista, New South Wales, Australia) and BP by finger photoplethysmography (Nexfin,

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3 136 BMEYE Medical Systems, Amsterdam, the Netherlands) were recorded with a sampling frequency of 1,000  
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5 137 Hz (PowerLab 8/35, ADInstruments). These preparations were followed by at least a 1-minute stabilization  
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7 138 period before the beginning of the 3-minute recording period in the seated position. After that recording period,  
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9 139 the participants stood up and remained still in a standing position for another 3 minutes while breathing  
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11 140 normally.

### 13 141 **Analysis of heart rate variability**

15 142 The first 150 seconds of recording in a seated position and the last 150 seconds in a standing position were  
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17 used in the analyses. The RRi data were edited based on visual inspections, and the artefacts and ectopic beats  
18 143 were removed and replaced according to the local average (Hearts 1.2, University of Oulu, Oulu, Finland).  
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20 144 Sequences with  $\geq 10$  consecutive beats of noise or ectopic beats were deleted. The RRi series with  $\geq 80\%$   
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22 145 accepted data were included in the analyses. The final study population included 1,029 controls and 160  
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24 146 women with PCOS. Mean heart rate, rMSSD (the square root of the mean squared differences of successive  
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26 147 normal-to-normal RRi) and spectral power densities (fast Fourier transformation, length 512 beats), including  
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28 148 low frequency (LF: 0.04–0.15 Hz,  $\text{ms}^2$ ) and high frequency (HF: 0.15–0.40 Hz,  $\text{ms}^2$ ) components of HRV and  
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30 149 their ratio (LF/HF), were analysed. Low frequency component reflects both the sympathetic and  
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32 150 parasympathetic activity, whereas the HF component mainly describes parasympathetic activity. [15]  
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### 36 152 **Analysis of baroreflex sensitivity**

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38 153 Baroreflex sensitivity (BRS) was assessed in the participants who had the measures performed at the Oulu  
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40 University Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals  
41 154 were imported to custom-made, stand-alone Matlab-based software (Biosignal Processing Team, University  
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43 155 of Oulu, Oulu, Finland), with which RRi and SBP values were extracted. Artefacts and ectopic beats were  
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45 156 replaced using linear interpolation ( $< 5\%$  for accepted recording) and, thereafter, resampled at 2 Hz and  
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47 157 detrended ( $< 0.04$  Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128  
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49 158 samples with 50% overlap) was performed to analyse the LF (0.04–0.15 Hz) power of RRi and SBP  
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51 159 oscillations for subsequent analysis of BRS using the alpha method if sufficient coherence ( $\geq 0.5$ ) between LF  
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53 160 oscillations in RRi and SBP was verified. The present BRS method quantifies cardiac autonomic responses to  
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55 161 spontaneous SBP variation, detected by baroreceptors in the aortic arch and the carotid sinus, which include  
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57 162 both parasympathetic and sympathetic effects.[24] Concurrently, the LF oscillation of blood pressure ( $\text{LF}_{\text{SBP}}$ ,  
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164 0.04–0.15 Hz) was obtained and considered as a surrogate for peripheral sympathetic activity. However, the  
165 physiological background of  $LF_{SBP}$  is not fully established, as there are competing theories of central oscillation  
166 of sympathetic drive and BRS resonance.[25]

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### 167 **Laboratory methods**

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168 The laboratory methods have previously been described in detail.[17] At age 46, sex hormone binding globulin  
169 (SHBG) was assayed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Llanberis,  
170 UK). The serum samples for testosterone (T) were assayed using Agilent triple quadrupole 6410 LC/MS  
171 equipment (Agilent Technologies, Wilmington, DE, USA). The free androgen index (FAI) was calculated  
172 using the following equation:  $(100 \times T) / SHBG$ . The serum total cholesterol, the high-density lipoprotein  
173 cholesterol (HDL), the low-density lipoprotein cholesterol (LDL) and triglycerides were determined using an  
174 enzymatic assay method. Fasting plasma glucose (f-gluc) was analysed by an enzymatic dehydrogenase  
175 method (methods of cholesterol, HDL, LDL, triglycerides, and f-gluc: Advia 1800, Siemens Healthcare  
176 Diagnostics Inc., Tarrytown, NY, USA). Fasting serum insulin (f-ins) was analysed by a chemiluminometric  
177 immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The f-gluc and f-  
178 ins values were used to calculate the Homeostasis Model Assessment–insulin resistance (HOMA–IR) index  
179  $(f\text{-gluc} \times f\text{-ins} / 22.5)$ . The high sensitivity C-reactive protein (hsCRP) was analysed by an immune-  
180 nephelometric assay (BN ProSpec, Siemens Healthcare Diagnostics Inc., Newark, DE, USA). The samples  
181 were analysed at NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service  
182 (FINAS) (EN ISO 15189).

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### 183 **Hopkins Symptom Check List-25**

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184 Hopkins Symptom Check List-25, a well-known and widely used symptom inventory, was used in the  
185 screening for anxiety. Part 1 includes 10 items that check for anxiety symptoms, and this part was used in the  
186 present study.[26]

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### 187 **Statistical methods**

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188 Women using beta-blockers (104 controls [7.7%] and 30 women with PCOS [13.3%],  $P=0.009$ ) were excluded  
189 from the HRV analysis. Continuous data were presented as mean with standard deviation or as median with  
190 25% and 75% quartiles. Continuous variables with skewed distributions were transformed into a natural  
191 logarithm (ln). Differences in normally distributed continuous parameters were analysed using the Student's

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3 192 *t*-test, whereas the Mann-Whitney *U*-test was used in the case of skewed distribution. Categorical data were  
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5 193 reported as prevalence with the number of cases, and the difference between the study groups was analysed by  
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7 194 cross-tabulation and the chi-square test or the Fisher's exact test, when appropriate. The mean arterial pressure  
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10 195 (MAP) was calculated as follows:  $DBP + \frac{1}{3}(SBP - DBP)$ .

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12 196 Univariate and multivariate linear regression analyses were used to study the factors associated  
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14 197 with the HRV parameters. First, univariate linear regression models were used to reveal the parameters that  
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16 198 were significantly associated with the outcome variable. Then, stepwise multivariate models were used to  
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18 199 identify the most important explanatory variables. The final multivariate model included the following  
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20 200 variables as explanatory variables: PCOS, BMI at age 46, MAP, FAI, HOMA-IR and triglycerides. The  
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22 201 number of explanatory variables included in the final model had to be limited to avoid multicollinearity. Body  
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24 202 mass index was included in the model, as it significantly differs between the PCOS and control women, and  
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26 203 obesity is suggested to affect HRV.[4] Mean arterial pressure was selected because it combines information  
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28 204 from both SBP and DBP, and FAI was included in the model because it is considered a good indicator of  
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30 205 hyperandrogenemia in women with PCOS,[2] and hyperandrogenemia has been suggested to alter HRV in  
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32 206 women with PCOS.[14] The homeostasis model assessment for insulin resistance was used as an estimate of  
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34 207 insulin resistance, as it combines information from both fasting insulin and glucose levels, and triglycerides  
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36 208 was included, as hypertriglyceridemia is a typical lipid abnormality in PCOS women and is linked to  
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38 209 cardiovascular disease risks.[27] Anxiety was not included in the final multivariate model, because in the  
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40 210 preliminary models using the stepwise method, it was always the first variable to be excluded. The results of  
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42 211 linear regression models are reported as unstandardized coefficients (B), 95% confidence intervals for B, *P*-  
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44 212 values, and *R*<sup>2</sup> values for the model. The multicollinearity assumptions of the multivariate linear regression  
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46 213 model were investigated using VIF, tolerance and eigenvalue indexes. In addition, a histogram of regression  
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48 214 standardized residual frequency, normal P-P plot of regression-standardized residuals, and scatter plot figures  
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50 215 were visually inspected to ensure that the model met the assumptions in the analysis. The data were analysed  
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52 216 using SPSS software (IBM SPSS Statistics 24.0, IBM Corp., New York, USA). A *P*-value < 0.05 was  
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54 217 considered statistically significant.

## 58 218 **Ethical approval**



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219 The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern  
220 Ostrobothnia Hospital District approved the research. All the participants took part on a voluntary basis and  
221 signed informed consent forms.

222 **Patient and public involvement statement**

223 The patients, the public or any third parties were not involved in the design, conduct, reporting or dissemination  
224 of our research.

For peer review only

## RESULTS

### Heart rate variability and baroreflex sensitivity

When compared with the control women, the women with PCOS in a seated position had a significantly higher mean HR and significantly lower values of rMSSD, LF<sub>RRi</sub>, HF<sub>RRi</sub> and baroreflex sensitivity (Figure 2). However, when adjusting for BMI at age 46, the women with PCOS had lower values only in rMSSD ( $P=0.033$ ) and HF<sub>RRi</sub> ( $P=0.016$ ) compared with the controls. In the standing position, the women with PCOS did not differ from the controls after adjustment for BMI at age 46 (data not shown).

### Heart rate variability according to BMI group

After dividing the women according to BMI ( $<25$  or  $\geq 25$  kg/m<sup>2</sup>), the lean women with PCOS did not differ from the lean control women regarding the HRV parameters, whereas the overweight/obese women with PCOS had higher HRs and LF/HF ratios and lower rMSSD, LF<sub>RRi</sub> and HF<sub>RRi</sub> compared to the overweight/obese controls (Figure 3). It is noteworthy that the overweight/obese women with PCOS had higher BMIs and waist circumferences and had abnormal glucose metabolism and hyperandrogenemia more often than the overweight/obese control women (data not shown).

We also further divided the population into overweight (BMI 25–30 kg/m<sup>2</sup>) and obese (BMI >30 kg/m<sup>2</sup>) groups. We found that the overweight women with PCOS ( $n=55$ ) had significantly lower rMSSD ( $2.54\pm 0.6$  vs.  $2.90\pm 0.6$ ,  $P=0.006$ ), LF<sub>RRi</sub> ( $4.91\pm 0.9$  vs.  $5.52\pm 0.9$ ,  $P=0.034$ ) and HF<sub>RRi</sub> ( $4.30\pm 1.2$  vs.  $5.00\pm 1.2$ ,  $P=0.010$ ) compared to the overweight control women ( $n=328$ ), whereas HR ( $80.0\pm 11.2$  vs.  $75.8\pm 9.6$ ,  $P=0.064$ ), LF/HF ratio ( $0.61\pm 0.9$  vs.  $0.51\pm 0.9$ ,  $P=0.187$ ), LF<sub>SBP</sub> ( $1.51\pm 0.8$  vs.  $1.80\pm 0.8$ ,  $P=0.087$ ), BRS ( $1.67\pm 0.4$  vs.  $1.81\pm 0.5$ ,  $p=0.182$ ) and  $\alpha$  ( $1.68\pm 0.4$  vs.  $1.81\pm 0.5$ ,  $P=0.182$ ) did not differ between these two groups. We found no significant differences between the obese PCOS and control women in any HRV parameters, but this might be due to a lack of statistical power, as our sample included only 23 obese women with PCOS (data not shown).

### Linear regression analysis for rMSSD

The univariate linear regression analysis demonstrated that rMSSD was associated with BMI at ages 31 and 46 and with waist circumference, anxiety, SBP, DBP, MAP and the serum levels of total cholesterol, HDL, LDL, triglycerides, glucose, insulin, HOMA-IR, SHBG, FAI and hsCRP at age 46 (Table 1). The body mass

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252 index at age 14 was not associated with rMSSD. The multivariate linear regression analysis demonstrated that  
 253 MAP, HOMA-IR and triglycerides were the strongest explanatory variables for rMSSD (Table 1).

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255 **Table 1.** Univariate and multivariate linear regression models for heart rate variability measure (rMSSD) in  
 256 women with PCOS

Variable	Univariate regression analysis				Multivariate Model* ( $B=4.075$ , $R^2=0.101$ )		
	B	95%CI for B	P-value	R <sup>2</sup>	B	95%CI for B	P-value
PCOS	NA	NA	NA	NA	-0.087	-0.190 – 0.016	0.099
BMI 14yr	-0.011	-0.022 – 0.000	0.057	0.002			
BMI 31yr	-0.022	-0.028 – -0.016	<0.001	0.022			
BMI 46yr	-0.028	-0.033 – -0.023	<0.001	0.055	-0.007	-0.016 – 0.002	0.140
Waist circumference	-0.012	-0.014 – -0.010	<0.001	0.061			
Systolic BP	-0.007	-0.009 – -0.005	<0.001	0.032			
Diastolic BP	-0.015	-0.017 – -0.013	<0.001	0.066			
Mean arterial pressure	-0.012	-0.014 – -0.010	<0.001	0.054	-0.007	-0.011 – -0.004	<0.001
Testosterone	0.042	-0.010 – 0.093	0.113	0.001			
SHBG	0.001	0.001 – 0.002	<0.001	0.005			
FAI	-0.121	-0.199 – -0.042	0.003	0.003	0.059	-0.055 – 0.173	0.309
Glucose	-0.121	-0.153 – -0.089	<0.001	0.023			
Insulin	-0.014	-0.018 – -0.011	<0.001	0.027			
HOMA-IR	-0.492	-0.585 – -0.399	<0.001	0.049	-0.256	-0.420 – -0.092	0.002
Total cholesterol	-0.067	-0.096 – -0.038	<0.001	0.009			
High-density lipoprotein	0.138	0.076 – 0.201	<0.001	0.008			
Low-density lipoprotein	-0.086	-0.114 – -0.058	<0.001	0.015			
Triglycerides	-0.243	-0.285 – -0.200	<0.001	0.050	-0.341	-0.558 – -0.123	0.002
High sensitive CRP	-0.071	-0.084 – -0.057	<0.001	0.034			
Anxiety (HSCL-25)	-0.195	-0.274 – -0.117	<0.001	0.010			

257 NA: not applicable. BMI: body mass index, BP: blood pressure, SHBG: sex hormone binding globulin, FAI:  
 258 free androgen index, HOMA-IR: homeostasis model assessment–insulin resistance, CRP: C-reactive protein.

259 \* The multivariate model included PCOS, BMI, mean arterial pressure, FAI, HOMA-IR and triglycerides as  
 260 explanatory variables. B: unstandardized coefficient from linear regression analysis, 95% CI: 95% confidence  
 261 interval.

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3 262 **Hyperandrogenemia**  
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5 263 Total or calculated free T at age 46 did not associate with rMSSD in the linear regression analysis (data not  
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7 264 shown). The FAI was negatively associated with rMSSD (B= -0.121, 95% CI: -0.199 – -0.042, *P*=0.003), but  
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9 265 lost its significance after an adjustment for BMI. Similarly, the serum level of SHBG was positively associated  
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11 266 with rMSSD (B=0.001, 95% CI: 0.001 – 0.002, *P*<0.001), but lost its significance after a BMI adjustment.  
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## 267 **DISCUSSION**

268 To our knowledge, this is the first study to investigate the cardiac autonomic function of late reproductive age  
269 women with PCOS. We demonstrate here, in a large general population-based setup, that late reproductive  
270 aged women with PCOS display reduced HRV, indicating reduced parasympathetic activity. However, in the  
271 multivariate linear regression analysis, the reduced HRV was associated with elevated BP, insulin resistance  
272 and dyslipidemia, but not PCOS *per se*, demonstrating that metabolic abnormalities are likely to be the main  
273 cause for reduced HRV in women with PCOS.

274 Previous studies have reported that women with PCOS display impaired cardiac autonomic  
275 function with decreased parasympathetic and increased sympathetic activity.[10-12] In line with our findings,  
276 a study of 75 overweight women with PCOS and 75 age- and BMI-matched controls suggested that impaired  
277 HR recovery, a marker for decreased parasympathetic activity, was caused by excess weight and insulin  
278 resistance and not by PCOS *per se*. [28] Furthermore, a cross-sectional study of 31 PCOS cases recruited from  
279 outpatient clinics reported that women with PCOS showed significantly decreased vagal activity, but the role  
280 of confounding metabolic abnormalities was not assessed, even though the women with PCOS had  
281 significantly higher BMIs, waist-hip ratios, BP, and serum levels of T and glucose than the control women.[10]  
282 Our findings are also in line with previous studies conducted in the general population in which an association  
283 between BP, glucose metabolism, dyslipidemia and cardiac autonomic function was reported.[5, 29]

284 In the present study, the overweight/obese women with PCOS had more adverse changes in  
285 HRV parameters than the overweight/obese control women, whereas the lean PCOS and control groups had  
286 comparable HRV values. However, the difference between the overweight/obese PCOS and control groups  
287 may have reflected the higher mean BMIs and waist circumferences and the higher prevalence of abnormal  
288 glucose metabolism in the overweight/obese PCOS group than in the overweight/obese controls. Previous  
289 studies have reported conflicting results regarding the effect of BMI on autonomic function in PCOS. A study  
290 of 19 overweight/obese women with PCOS and 21 overweight/obese control women reported that the women  
291 with PCOS had elevated muscle sympathetic nervous activity (MSNA), whereas HRV parameters did not  
292 significantly differ between the groups.[13] However, it was reported that the non-obese (BMI<30kg/m<sup>2</sup>)  
293 control and PCOS groups did not have significantly different standard deviation of all RRis (SDNN), rMSSD  
294 or percentage of successive differences in RRi > 50 ms (pNN50), whereas the obese (BMI≥30 kg/m<sup>2</sup>) women

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3 295 with PCOS had significantly decreased SDNN and pNN50, but not rMSSD.[30] The findings of that study  
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5 296 also demonstrated that sympathetic skin responses, investigated by electromyography from the median or tibial  
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7 297 nerve, were altered in both the non-obese and obese PCOS groups. These conflicting results might be explained  
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9 298 by the fact that the effect of obesity in sympathetic activation might be regional, as obesity has been reported  
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11 299 to increase sympathetic activity in the kidneys and skeletal muscle vasculature but to reduce it in the heart.[31]

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14 300 We found that serum T or FAI at age 46 did not associate with HRV after BMI adjustment (nor  
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16 301 did FAI or T at age 31, data not shown). In line with our findings, two previous studies found no significant  
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18 302 association between T and LF [12] or hormonal profile and HR recovery.[11] By contrast, the total T level was  
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20 303 reported to be inversely associated with LF and LF/HF ratio in the women with PCOS during mental stress  
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22 304 testing.[32] However, in the present study, we used the golden standard, a liquid-tandem mass-spectrometry  
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24 305 assay for T measurement, whereas the previous studies have used immunoassays.[11, 32] Another study found  
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26 306 higher MSNA in the normal weight women with PCOS compared with the normal weight controls, and the  
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28 307 strongest explanatory factors for higher MSNA in the women with PCOS were total and free T and  
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30 308 cholesterol.[14] However, MSNA describes sympathetic activity, whereas HRV mainly describes  
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33 309 parasympathetic activity.

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35 310 Also, the phenotype of PCOS was suggested to influence cardiac autonomic function, as the  
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37 311 anovulatory women with PCOS showed lower HRV response in mental stress tests than the controls, whereas  
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39 312 the ovulatory PCOS women showed intermediate values.[32] However, in that study, the women with  
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41 313 anovulatory PCOS had a different metabolic profile than those with ovulatory PCOS and the control  
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43 314 groups.[32] Furthermore, previous studies have indicated that anxiety was associated with reduced HRV in the  
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45 315 general population.[8] In our analysis, anxiety had a weak association with rMSSD in the univariate linear  
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47 316 regression model, although a further analysis revealed that metabolic abnormalities played a more important  
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49 317 role in the reduction of vagal activity.

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51 318 A recent study addressed the interrelated effects of insulin resistance, hyperandrogenism,  
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53 319 chronic inflammation and sympathetic dysfunction (evaluated by MSNA) in 49 PCOS and 23 control women;  
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55 320 based on the findings, the authors concluded that sympathetic dysfunction and hyperandrogenism were  
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57 321 associated with PCOS and that chronic inflammation might be the mediating factor between sympathetic  
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59 322 function, hyperandrogenism and insulin resistance.[33]. However, in the present study, the surrogate marker

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323 of sympathetic activity,  $LF_{SBP}$ , did not significantly differ between the PCOS and control women, suggesting  
324 that in our population, the women with PCOS would not have increased sympathetic activity. This needs to be  
325 interpret with caution as  $LF_{SBP}$  does not directly measure sympathetic activity. The frequency width of 0.04–  
326 0.15Hz used in the present study could be considered a limitation, as a frequency width of 0.075–0.15 Hz is  
327 affected by sympathetic modulation.[34] However, the LF oscillation of BP usually has a central frequency at  
328 ~0.1 Hz that considerably varies in relation to sympathetic effect, [35-36] supporting the use of a wider spectral  
329 band for  $LF_{SBP}$ . Of note, in our data, the women with PCOS had significantly lower BRS, but the significance  
330 disappeared after adjusting for BMI, indicating that PCOS *per se* does not affect BRS.

331 The strength of our study is that it includes by far the largest sample size of women with PCOS  
332 and HRV measurements. The data also add to the literature by representing a community-based approach.  
333 Moreover, we were able to adjust for many confounding factors and to study the effect of metabolic  
334 abnormalities. Also, this is the first study to investigate women with PCOS at a late fertile age. The definition  
335 of the PCOS population could be considered a limitation; however, we have previously shown that the  
336 population does display the typical endocrine, metabolic and psychological profiles of PCOS.[21-23]  
337 Moreover, a recent genome-wide meta-analysis reported that the genetic architecture does not differ based on  
338 the diagnostic criteria used for PCOS (self-reported, NIH criteria or non-NIH Rotterdam criteria),[37] thus  
339 supporting our approach. Our study population included only women with Caucasian ethnicity; consequently,  
340 our results are best generalized to PCOS women with Caucasian ethnicity, as ethnicity is known to affect many  
341 traits of PCOS. In addition, a longer HRV recording period would have been more favorable, but due to  
342 logistical reasons and the large number of subjects to measure, we had to limit the time. However, the recording  
343 period of 150 second used in the present study should be reliable.[15]

344 In conclusion, in this community-based data set, the women with PCOS displayed reduced vagal  
345 activity at a late fertile age with a strong association with metabolic abnormalities. The fact is that impaired  
346 cardiovascular autonomic function (i.e. increased sympathetic/decreased parasympathetic activity) reflects the  
347 risk for cardiac morbidity; this is most likely also the case in PCOS. This underlines the importance of the  
348 active screening and treatment of metabolic abnormalities in women with PCOS, as also suggested by the  
349 recently published guidelines for PCOS.[38] Previous studies have reported that in overweight/obese women  
350 with PCOS, the impaired cardiovascular autonomic function could be improved by a 10-week energy

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3 351 restriction,[39] a 3-month aerobic exercise training program[40] or acupuncture.[41] Importantly, it has been  
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5 352 shown that the autonomic disturbance can be reversed with weight reduction,[42] which should justify  
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7 353 increasing resources and efforts targeting weight management.  
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11 355 **A DATA STATEMENT:** The data are available on request to the NFBC1966 Data Sharing Committee.  
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13 356 NFBC1966 data sharing policies and processes meet the requirement and expectations of the Northern  
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15 357 Ostrobothnia Hospital district policy on sharing of data from population and patient cohorts. Further  
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17 358 information can be found at <https://www.oulu.fi/nfbc/>.

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21

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46

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49 373 and revision; AK: study design, data collection, analyses and interpretation, manuscript drafting and revision;  
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51 374 ESV: data interpretation, manuscript drafting and revision; MT: data collection and interpretation, manuscript  
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53 375 revision; KP: laboratory analyses and manuscript revision; JST: data interpretation and manuscript revision;  
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55 376 SF: data interpretation and manuscript revision; LMP: data interpretation, manuscript drafting and revision;  
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57 377 and TTP: study design, data interpretation, manuscript drafting and revision.  
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60**REFERENCES**

- 1 March WA, Moore VM, Willson KJ, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;25:544–51. doi: 10.1093/humrep/dep399.
- 2 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25. doi: 10.1016/j.fertnstert.2003.10.004
- 3 Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;333:853–61. doi: 10.1056/NEJM199509283331307
- 4 Lambert GW, Straznicky NE, Lambert EA, et al. Sympathetic nervous activation in obesity and the metabolic syndrome--causes, consequences and therapeutic implications. *Pharmacol Ther* 2010;126:159–72. doi:10.1016/j.pharmthera.2010.02.002.
- 5 Stuckey MI, Tulppo MP, Kiviniemi AM, et al. Heart rate variability and the metabolic syndrome: a systematic review of the literature. *Diabetes Metab Res Rev* 2014;30:784–93 doi:10.1002/dmrr.2555.
- 6 Kakoly NS, Moran LJ, Teede HJ, et al. Cardiometabolic risks in PCOS: a review of the current state of knowledge. *Expert Rev Endocrinol Metab* 2019;14:23–33. doi: 10.1080/17446651.2019.1556094.
- 7 Sgoifo A, Carnevali L, Alfonso Mde L, et al. Autonomic dysfunction and heart rate variability in depression. *Stress* 2015;18:343–352 doi:10.3109/10253890.2015.1045868.
- 8 Paniccia M, Paniccia D, Thomas S, et al. Clinical and non-clinical depression and anxiety in young people: A scoping review on heart rate variability. *Auton Neurosci* 2017;208:1–14 doi:10.1016/j.autneu.2017.08.008.
- 9 Wulsin LR, Horn PS, Perry JL, et al. Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality. *J Clin Endocrinol Metab* 2015;100:2443–2448 doi:10.1210/jc.2015-1748.
- 10 Saranya K, Pal GK, Habeebullah S, et al. Assessment of cardiovascular autonomic function in patients with polycystic ovary syndrome. *J Obstet Gynaecol Res* 2014;40:192–199 doi:10.1111/jog.12154.
- 11 Tekin G, Tekin A, Kiliçarslan EB, et al. Altered autonomic neural control of the cardiovascular system in patients with polycystic ovary syndrome. *Int J Cardiol* 2008;130:49–55 doi:10.1016/j.ijcard.2007.08.037.
- 12 Yildirim A, Aybar F, Kabakci G, et al. Heart rate variability in young women with polycystic ovary syndrome. *Ann Noninvasive Electrocardiol* 2006;11:306–312 doi:10.1111/j.1542-474X.2006.00122.x.

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- 13 Lambert EA, Teede H, Sari CI, et al. Sympathetic activation and endothelial dysfunction in polycystic ovary syndrome are not explained by either obesity or insulin resistance. *Clin Endocrinol (Oxf)* 2015;83:812–819 doi:10.1111/cen.12803.
- 14 Sverrisdóttir YB, Mogren T, Kataoka J, et al. Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth? *Am J Physiol Endocrinol Metab* 2008;294:576–581 doi:10.1152/ajpendo.00725.2007.
- 15 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996;93:1043–1065.
- 16 Brown ZA, Louwers YV, Fong SL, et al. The phenotype of polycystic ovary syndrome ameliorates with aging. *Fertil Steril* 2011;96:1259–1265 doi:10.1016/j.fertnstert.2011.09.002.
- 17 Ollila MM, Piltonen T, Puukka K, et al. Weight gain and dyslipidemia in early adulthood associate with polycystic ovary syndrome: prospective cohort study. *J Clin Endocrinol Metab* 2016;101:739–747 doi:10.1210/jc.2015-3543.
- 18 Ollila ME, Kaikkonen K, Järvelin M, et al. Self-reported polycystic ovary syndrome is associated with hypertension: a Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* 2019;104:1221–1231 doi:10.1210/jc.2018-00570.
- 19 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539–553.
- 20 Ollila MM, West S, Keinänen-Kiukaanniemi S, et al. Overweight and obese but not normal weight women with PCOS are at increased risk of Type 2 diabetes mellitus—a prospective, population-based cohort study. *Hum Reprod* 2017;32:423–431. doi: 10.1093/humrep/dew329.
- 21 Karjula S, Morin-Papunen L, Auvinen J, et al. Psychological distress is more prevalent in fertile age and premenopausal women with PCOS symptoms: 15-year follow-up. *J Clin Endocrinol Metab* 2017;102:1861–1869 doi: 10.1210/jc.2016-3863.

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- 432 22 Taponen S, Martikainen H, Järvelin MR, et al. Hormonal profile of women with self-reported symptoms of  
433 oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab*  
434 2003;88:141–147. doi: 10.1210/jc.2002-020982
- 435 23 Taponen S, Ahonkallio S, Martikainen H, et al. Prevalence of polycystic ovaries in women with self-  
436 reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland birth cohort 1966 study. *Hum*  
437 *Reprod* 2004;19:1083–1088. doi: 10.1093/humrep/deh214.
- 438 24 Pagani M, Somers V, Furlan R, et al. Changes in autonomic regulation induced by physical training in mild  
439 hypertension. *Hypertension* 1988;12:600–610 doi:10.1161/01.hyp.12.6.600.
- 440 25 Julien C. The enigma of Mayer waves: Facts and models. *Cardiovasc Res* 2006;70:12–21  
441 doi:10.1016/j.cardiores.2005.11.008.
- 442 26 Veijola J, Jokelainen J, Läksy K, et al. The Hopkins Symptom Checklist-25 in screening DSM-III-R axis-  
443 I disorders. *Nord J Psychiatry* 2003;57:119–123 doi:10.1080/08039480310000941.
- 444 27 Fauser BC, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary  
445 syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil*  
446 *Steril* 2012;97:28–38.e25. doi: 10.1016/j.fertnstert.2011.09.024.
- 447 28 Giallauria F, Palomba S, Manguso F, et al. Abnormal heart rate recovery after maximal cardiopulmonary  
448 exercise stress testing in young overweight women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
449 2008;68:88–93 doi:10.1111/j.1365-2265.2007.03004.x.
- 450 29 Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability  
451 and cardiovascular disease risk factors. *Int J Cardiol* 2010;141:122–131 doi:10.1016/j.ijcard.2009.09.543.
- 452 30 Hashim ZH, Hamdan FB, Al-Salihi AR. Autonomic dysfunction in women with polycystic ovary syndrome.  
453 *Iran J Reprod Med* 2015;13:27–34.
- 454 31 Vaz M, Jennings G, Turner A, et al. Regional sympathetic nervous activity and oxygen consumption in  
455 obese normotensive human subjects. *Circulation* 1997;96:3423–3429 doi:10.1161/01.cir.96.10.3423.
- 456 32 Di Domenico K, Wiltgen D, Nickel FJ, et al. Cardiac autonomic modulation in polycystic ovary syndrome:  
457 does the phenotype matter? *Fertil Steril* 2013;99:286–292 doi:10.1016/j.fertnstert.2012.08.049.

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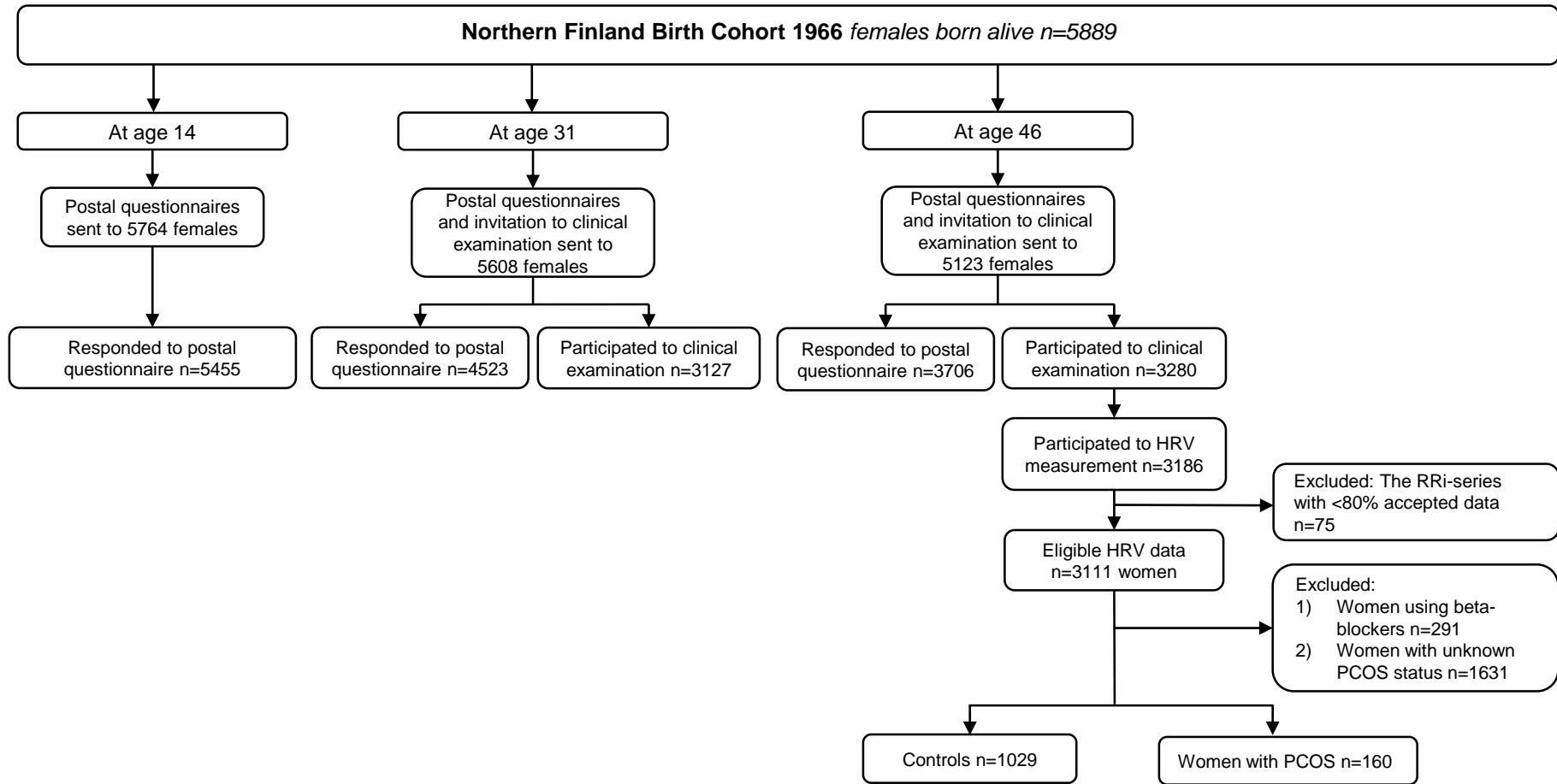
- 458 33 Shorakae S, Ranasinha S, Abell S, et al. Inter-related effects of insulin resistance, hyperandrogenism,  
459 sympathetic dysfunction and chronic inflammation in PCOS. *Clin Endocrinol (Oxf)* 2018;89:628–633  
460 doi:10.1111/cen.13808.
- 461 34 Stauss HM. Identification of blood pressure control mechanisms by power spectral analysis. *Clin Exp*  
462 *Pharmacol Physiol* 2007;34:362–368 doi:10.1111/j.1440-1681.2007.04588.x.
- 463 35 Malliani A, Pagani M, Lombardi F, et al. Cardiovascular neural regulation explored in the frequency  
464 domain. *Circulation* 1991;84:482–492 doi:10.1161/01.cir.84.2.482.
- 465 36 Kiviniemi AM, Frances MF, Tiinanen S, et al.  $\alpha$ -Adrenergic effects on low-frequency oscillations in blood  
466 pressure and R-R intervals during sympathetic activation. *Exp Physiol* 2011;96:718–735  
467 doi:10.1113/expphysiol.2011.058768.
- 468 37 Day F, Karaderi T, Jones MR, et al. Large-scale genome-wide meta-analysis of polycystic ovary syndrome  
469 suggests shared genetic architecture for different diagnosis criteria. *PLoS Genet* 2018;14:e1007813  
470 doi:10.1371/journal.pgen.1007813.
- 471 38 Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based  
472 guideline for the assessment and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
473 2018;89:251–268. doi: 10.1111/cen.13795.
- 474 39 Thomson RL, Buckley JD, Noakes M, et al. Heart rate recovery improves after weight loss in overweight  
475 and obese women with polycystic ovary syndrome. *Fertil Steril* 2010;93:1173–1178  
476 doi:10.1016/j.fertnstert.2008.12.003.
- 477 40 Giallauria F, Palomba S, Maresca L, et al. Exercise training improves autonomic function and inflammatory  
478 pattern in women with polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2008;69:792–798  
479 doi:10.1111/j.1365-2265.2008.03305.x.
- 480 41 Stener-Victorin E, Jedel E, Janson PO, et al. Low-frequency electroacupuncture and physical exercise  
481 decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am J Physiol Regul Integr*  
482 *Comp Physiol* 2009;297:387–395. doi:10.1152/ajpregu.00197.2009.
- 483 42 Maser RE, Lenhard MJ. An overview of the effect of weight loss on cardiovascular autonomic function.  
484 *Curr Diabetes Rev* 2007;3:204–211.

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60**FIGURE LEGENDS**

**Figure 1.** Flow chart of the study. RRI: R-R interval.

**Figure 2.** Heart rate variability parameters in controls and in women with PCOS at age 46 in seated position. The users of beta-blockers were excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles, and the significance testing was made by Student's *t*-test (ln-transform was made to achieve normality). HR: heart rate. rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals (RRI), LF<sub>RRI</sub>: low frequency (0.04–0.15 Hz) power, HF<sub>RRI</sub>: high frequency (0.15–0.4 Hz) power,  $\alpha_1$ : short-term fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex sensitivity.

**Figure 3.** Heart rate variability parameters in controls and in women with PCOS at age 46 according to the BMI group. The assessment of autonomic function in seated position. The users of beta blockers were excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles, and the significance testing was made by Student's *t*-test (ln-transform was made to achieve normality). Statistically significant *P*-values are bolded. rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals (RRI), LF<sub>RRI</sub>: low frequency (0.04-0.15 Hz) power, HF<sub>RRI</sub>: high frequency (0.15-0.4 Hz) power,  $\alpha_1$ : short-term fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex sensitivity.



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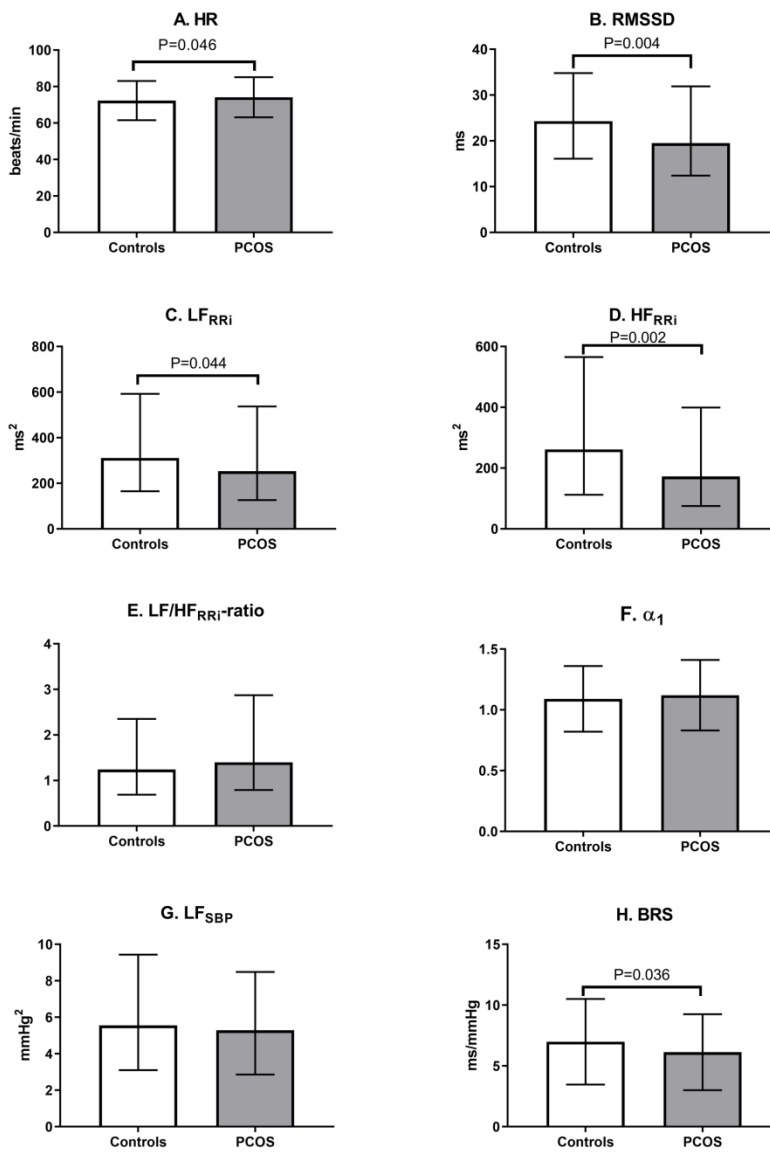


Figure 2

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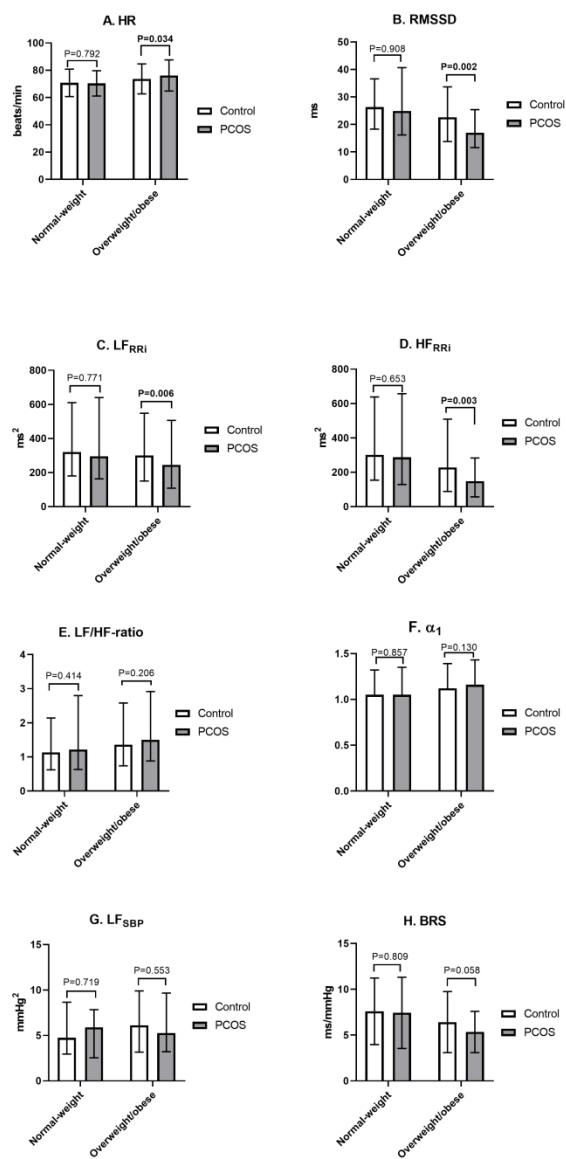


Figure 3

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STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	Page 1,2	
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4	
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4	
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	Page 5 and 6	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5 and 6	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	Page 5 and 6	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pages 6 – 8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 6 – 8	
Bias	9	Describe any efforts to address potential sources of bias	Page 15	
Study size	10	Explain how the study size was arrived at	Page 5 and 6	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 8 and 9
		(b) Describe any methods used to examine subgroups and interactions	Page 8 and 9
		(c) Explain how missing data were addressed	NA
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	NA
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pages 5 – 7
		(b) Give reasons for non-participation at each stage	Pages 5 – 7
		(c) Consider use of a flow diagram	Flow chart is included
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	Page 5
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 9 – 12
		(b) Report category boundaries when continuous variables were categorized	Pages 9 – 10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Pages 13–15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 13–15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 15
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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

# BMJ Open

## THE EFFECT OF POLYCYSTIC OVARY SYNDROME ON CARDIAC AUTONOMIC FUNCTION AT A LATE FERTILE AGE: A PROSPECTIVE NORTHERN FINLAND 1966 BIRTH COHORT STUDY

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<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Cardiovascular medicine, Reproductive medicine
Keywords:	PCOS, Heart rate, Heart rate variability, Obesity, Metabolism

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13 5 Meri-Maija Ollila<sup>1</sup>, Antti M. Kiviniemi<sup>2</sup>, Elisabet Stener-Victorin<sup>3</sup>, Mikko Tulppo<sup>2</sup>, Katri Puukka<sup>4</sup>, Juha S.  
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**ABSTRACT**

**Objectives:** Previous studies of women in their 20s and 30s have reported impaired autonomic function in women with polycystic ovary syndrome (PCOS). We aimed to study, for the first time, whether PCOS is associated with impaired cardiac autonomic function independent of metabolic and hormonal status in their late reproductive years.

**Design:** A prospective Northern Finland birth cohort (NFBC66) study including 5,889 females born in 1966 and followed through the age of 46. At that age,  $n=3,706/5,123$  women (72%) answered the postal questionnaires and  $n=3,280/5,123$  women (64%) participated to the clinical examination.

**Setting:** General community.

**Participants:** The sample included women presenting both irregular menses (oligomenorrhea or amenorrhea) and hirsutism at age 31 ( $n=125$ ) or with formally diagnosed PCOS by age 46 ( $n=181$ ) and women without PCOS symptoms or diagnosis ( $n=1,577$ ).

**Primary and secondary outcome measures:** Heart rate variability parameters: the root mean square of successive R–R differences (rMSSD), spectral power densities (LF: low frequency and HF: high frequency), and baroreflex sensitivity (BRS).

**Results:** We found that parasympathetic activity (assessed by rMSSD: 19.5 [12.4; 31.9] vs. 24.3 [16.1; 34.8] ms,  $P=0.004$  and HF: 172 [75; 399] vs. 261 [112; 565] ms<sup>2</sup>,  $P=0.002$ ) and BRS ( $6.13\pm 3.12$  vs.  $6.99\pm 3.52$  ms/mmHg,  $P=0.036$ ) were lower in women with PCOS compared with the controls.

However, in the multivariate regression analysis, PCOS, body mass index and the free androgen index did not significantly associate with rMSSD, whereas blood pressure, insulin resistance and triglycerides did.

**Conclusions:** We report here for the first time that late reproductive-aged women with PCOS display impaired cardiac autonomic function manifested as decreased vagal activity. Metabolic status, rather

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16 52 **Keywords:** PCOS, heart rate, heart rate variability, obesity, metabolism  
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### 53 **Strengths and limitations of this study**

- 54 • This is the first study to investigate the cardiac autonomic function of late reproductive aged  
55 women with PCOS.
- 56 • This study provides the largest study population by far compared to the previous studies in  
57 cardiac autonomic function in women with PCOS.
- 58 • We were able to adjust for many confounding factors and to study the effect of metabolic  
59 abnormalities.
- 60 • The study is limited by the lack of PCOS phenotypes.
- 61 • The study cannot be generalized with all ethnicities.

## 62 INTRODUCTION

63 Polycystic ovary syndrome (PCOS) is the most common endocrinopathy, affecting 6–18% of women  
64 of reproductive age and characterized by irregular menstruation, clinical or biochemical  
65 hyperandrogenism, and polycystic ovaries.[1-3] Women with PCOS are commonly overweight or  
66 obese and typically present with insulin resistance, hyperinsulinemia, increased blood pressure,  
67 dyslipidemia, metabolic syndrome and obstructive sleep apnea,[2] all of which are associated with  
68 impaired cardiac autonomic function.[4-6] In the general population, the dysregulation of cardiac  
69 autonomic function has been associated with increased risk of many major global public health  
70 problems, such as depression,[7] anxiety,[8] hypertension, diabetes, cardiovascular diseases and  
71 mortality.[9] Therefore, it is not surprising that women with PCOS have been shown to present with  
72 impaired cardiac autonomic function; that is, reduced parasympathetic (vagal) activity,[10-12] and  
73 increased sympathetic nervous system activity.[13, 14] Previous researchers have used various  
74 methods to assess the cardiac autonomic function in women with PCOS, such as microneurography,  
75 the measurement of sympathetic skin responses, heart rate (HR) variability (HRV), HR recovery,  
76 and a noradrenaline spill-over measurement. Of these methods, the measurement of HRV (i.e.  
77 variations in the time intervals between consecutive heartbeats), provides a well-established non-  
78 invasive method to assess, in particular, parasympathetic cardiac autonomic activity.[15]

79 During recent years, it has become an enigma whether women with PCOS have an increased  
80 risk for cardiovascular disease (CVD). Taking into consideration that many PCOS traits, such as irregular

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3 81 cycles, hyperandrogenism and body mass index (BMI) difference from non-PCOS controls, seem to diminish  
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5 82 with age,[16] it is also important to assess CVD-related traits, such as cardiac autonomic function, in women  
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7 83 with PCOS in the late reproductive years and beyond menopause to elucidate their possible risk for CVD  
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9 84 outcomes. Previous studies on cardiac autonomic function have included women with PCOS in their 20s or  
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11 85 30s, but to date, no studies have been carried out on women in their late reproductive years. Moreover, the  
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13 86 populations studied have been derived from PCOS clinics; thus, community-based studies are needed.  
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15 87 Therefore, the main aim of this study was to investigate whether women with PCOS from a general population  
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17 88 display reduced HRV as an indicator of impaired cardiac autonomic function during their late reproductive  
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19 89 years (at age 46). Additionally, we investigated the role of confounding metabolic abnormalities, such as  
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21 90 excess weight, abdominal obesity, hyperandrogenism, increased blood pressure (BP), dyslipidemia and insulin  
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23 91 resistance, in the cardiac autonomic function in affected women.  
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## 29 93 **MATERIALS AND METHODS**

### 30 94 **Study population**

31 95 The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large,  
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33 96 prospective, general population-based, longitudinal birth cohort. All individuals with expected births during  
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35 97 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort  
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37 98 (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began  
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39 99 at the 24<sup>th</sup> gestational week, and the women were followed through age 46. The follow-up protocol of the  
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41 100 cohort was previously described in detail, and the main data collection points during adulthood were carried  
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43 101 out at ages 31 and 46 years.[17] Briefly, postal questionnaires were sent to all living cohort members with  
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45 102 known addresses at ages 31 (81% answered,  $n=4,523/5,608$ ) and 46 (72% answered,  $n=3,706/5,123$ ) to collect  
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48 103 information about health, behaviour and social background. Postal questionnaires included an invitation to  
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50 104 participate in clinical examinations at age 31 (77% participation rate,  $n=3,127/4,074$ ) and at age 46 (64%  
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52 105 participation rate,  $n=3,280/5,123$ ). Weight and height were self-reported at age 14 (with the help of the  
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54 106 participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio  
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57 107 of weight (kg) and height squared ( $m^2$ ). Besides anthropometric measurements, the clinical examinations at  
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3 108 age 46 included blood sampling and assessments of cardiovascular health status, including systolic and  
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5 109 diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound, and evaluations of HRV  
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7 110 and baroreflex sensitivity. Waist circumference was measured at the level midway between the lowest rib  
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9 111 margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval after 15  
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11 112 minutes rest by an automated, oscillometric BP device with an appropriately sized cuff (Omron Digital  
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13 113 Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan), and SBP and DBP averages were  
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16 114 calculated.[18] The level of glucose metabolism was classified according to World Health Organization  
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18 115 standards,[19] based on a 2-hour oral glucose tolerance test (performed at age 46) and a previously established  
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20 116 diagnosis of type 2 diabetes.[20]

### 21 22 117 **Definition of PCOS and control groups**

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24 118 At age 31, PCOS symptoms (i.e. oligomenorrhea/amenorrhea and hirsutism) were self-reported. Of all the  
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26 119 women who responded to questions regarding PCOS symptoms ( $n=4,523$ ), after excluding pregnant women  
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28 120 and those using hormonal preparations ( $n=1,459$ ) or not permitting the use of their data ( $n=41$ ), 4.1% ( $n=125$ )  
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30 121 reported both oligomenorrhea/amenorrhea and hirsutism. The validity of this questionnaire to distinguish  
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32 122 PCOS cases with typical hormonal, metabolic and psychological traits characteristic to the syndrome, as well  
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35 123 as ovarian morphology for PCOS, has previously been described.[20-23] At age 46, the postal questionnaire  
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37 124 included a question on existing PCOS diagnosis, to which 181 subjects responded “yes.” Consequently, the  
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39 125 women reporting both oligomenorrhea/amenorrhea and hirsutism and/or reporting PCOS diagnosis by age 46  
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41 126 were considered cases ( $n=279$ ). Women without PCOS symptoms at age 31 and without diagnosis of PCOS  
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43 127 by age 46 were considered controls ( $n=1,577$ ). The characteristics of the PCOS and control populations and  
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45 128 the flow chart of the formation of the PCOS and control groups have previously been described.[17]

### 46 47 129 **Evaluation of cardiac autonomic function**

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49 130 A flow chart of the study is presented in Figure 1. Heart rate variability was measured in the study subjects at  
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51 131 age 46 in the research unit at Oulu University Hospital and in two other major hospitals nearby. The subjects  
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53 132 were informed about the measurement protocol, and an HR monitor (RS800CX, Polar Electro Oy, Kempele,  
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55 133 Finland) to record R-R intervals (RRi) and a standard lead-II ECG (Cardiolife, Nihon Kohden, Tokyo, Japan)  
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58 134 were placed on the subjects while seated. Also, breathing frequency (MLT415/D, Nasal Temperature Probe,  
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60 135 ADInstruments, Bella Vista, New South Wales, Australia) and BP by finger photoplethysmography (Nexfin,

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3 136 BMEYE Medical Systems, Amsterdam, the Netherlands) were recorded with a sampling frequency of 1,000  
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5 137 Hz (PowerLab 8/35, ADInstruments). These preparations were followed by at least a 1-minute stabilization  
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7 138 period before the beginning of the 3-minute recording period in the seated position. After that recording period,  
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9 139 the participants stood up and remained still in a standing position for another 3 minutes while breathing  
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11 140 normally.

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#### 14 141 **Analysis of heart rate variability**

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16 142 The first 150 seconds of recording in a seated position and the last 150 seconds in a standing position were  
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18 143 used in the analyses. The RRi data were edited based on visual inspections, and the artefacts and ectopic beats  
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20 144 were removed and replaced according to the local average (Hearts 1.2, University of Oulu, Oulu, Finland).  
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22 145 Sequences with  $\geq 10$  consecutive beats of noise or ectopic beats were deleted. The RRi series with  $\geq 80\%$   
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24 146 accepted data were included in the analyses. The final study population included 1,029 controls and 160  
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26 147 women with PCOS. Mean heart rate, rMSSD (the square root of the mean squared differences of successive  
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28 148 normal-to-normal RRi) and spectral power densities (fast Fourier transformation, length 512 beats), including  
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30 149 low frequency (LF: 0.04–0.15 Hz,  $ms^2$ ) and high frequency (HF: 0.15–0.40 Hz,  $ms^2$ ) components of HRV and  
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32 their ratio (LF/HF), were analysed. Whereas rMSSD and HF component of HRV are mainly determined by  
33 150 cardiac parasympathetic activity, LF component of HRV is affected largely by parasympathetic activity but  
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35 151 also includes effects of sympathetic activity as well as other unidentified factors. [15, 24] The LF/HF ratio has  
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37 152 been used as a marker of sympatho-vagal balance, particularly during orthostatic stimulus.[25] However, as  
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39 153 the physiological background of LF component of HRV is complex, the conclusions concerning sympatho-  
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41 154 vagal balance by LF/HF are limited.[24]

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#### 45 156 **Analysis of baroreflex sensitivity**

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47 157 Baroreflex sensitivity (BRS) was assessed in the participants who had the measures performed at the Oulu  
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49 158 University Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals  
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51 159 were imported to custom-made, stand-alone Matlab-based software (Biosignal Processing Team, University  
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53 160 of Oulu, Oulu, Finland), with which RRi and SBP values were extracted. Artefacts and ectopic beats were  
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55 replaced using linear interpolation ( $< 5\%$  for accepted recording) and, thereafter, resampled at 2 Hz and  
56 161 detrended ( $< 0.04$  Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128  
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58 162 samples with 50% overlap) was performed to analyse the LF (0.04–0.15 Hz) power of RRi and SBP  
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3 164 oscillations for subsequent analysis of BRS using the alpha method if sufficient coherence ( $\geq 0.5$ ) between LF  
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5 165 oscillations in RRi and SBP was verified. The present BRS method quantifies cardiac autonomic responses to  
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7 166 spontaneous SBP variation, detected by baroreceptors in the aortic arch and the carotid sinus, which include  
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9 167 both parasympathetic and sympathetic effects.[26] Concurrently, the LF oscillation of blood pressure ( $LF_{SBP}$ ,  
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11 168 0.04–0.15 Hz) was obtained and considered as a surrogate for peripheral sympathetic activity. However, the  
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14 169 physiological background of  $LF_{SBP}$  is not fully established, as there are competing theories of central oscillation  
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16 170 of sympathetic drive and BRS resonance.[27]

### 17 18 171 **Laboratory methods**

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20 172 The laboratory methods have previously been described in detail.[17] At age 46, sex hormone binding globulin  
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22 173 (SHBG) was assayed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Llanberis,  
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24 174 UK). The serum samples for testosterone (T) were assayed using Agilent triple quadrupole 6410 LC/MS  
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26 175 equipment (Agilent Technologies, Wilmington, DE, USA). The free androgen index (FAI) was calculated  
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28 176 using the following equation:  $(100 \times T) / SHBG$ . The serum total cholesterol, the high-density lipoprotein  
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30 177 cholesterol (HDL), the low-density lipoprotein cholesterol (LDL) and triglycerides were determined using an  
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33 178 enzymatic assay method. Fasting plasma glucose (f-gluc) was analysed by an enzymatic dehydrogenase  
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35 179 method (methods of cholesterol, HDL, LDL, triglycerides, and f-gluc: Advia 1800, Siemens Healthcare  
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37 180 Diagnostics Inc., Tarrytown, NY, USA). Fasting serum insulin (f-ins) was analysed by a chemiluminometric  
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39 181 immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The f-gluc and f-  
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41 182 ins values were used to calculate the Homeostasis Model Assessment–insulin resistance (HOMA–IR) index  
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43 183  $(f\text{-gluc} \times f\text{-ins} / 22.5)$ . The high sensitivity C-reactive protein (hsCRP) was analysed by an immune-  
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45 184 nephelometric assay (BN ProSpec, Siemens Healthcare Diagnostics Inc., Newark, DE, USA). The samples  
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47 185 were analysed at NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service  
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50 186 (FINAS) (EN ISO 15189).

### 51 52 187 **Hopkins Symptom Check List-25**

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54 188 Hopkins Symptom Check List-25 Part 1 includes 10 items that check for anxiety symptoms, and this part  
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56 189 was used in the present study.[28]

### 57 58 190 **Statistical methods**

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191 Women using beta-blockers (104 controls [7.7%] and 30 women with PCOS [13.3%],  $P=0.009$ ) were excluded  
192 from the HRV analysis. Continuous data were presented as mean with standard deviation or as median with  
193 25% and 75% quartiles. Continuous variables with skewed distributions were transformed into a natural  
194 logarithm (ln). Differences in normally distributed continuous parameters were analysed using the Student's  
195  $t$ -test, whereas the Mann-Whitney  $U$ -test was used in the case of skewed distribution. Categorical data were  
196 reported as prevalence with the number of cases, and the difference between the study groups was analysed by  
197 cross-tabulation and the chi-square test or the Fisher's exact test, when appropriate. The mean arterial pressure  
198 (MAP) was calculated as follows:  $DBP + \frac{1}{3}(SBP - DBP)$ .

199 Univariate and multivariate linear regression analyses were used to study the factors associated with the HRV  
200 parameters. First, univariate linear regression models were used to reveal the parameters that were significantly  
201 associated with the outcome variable. Then, stepwise multivariate models were used to identify the most  
202 important explanatory variables. The final multivariate model included the following variables as explanatory  
203 variables: PCOS, BMI at age 46, MAP, FAI, HOMA-IR and triglycerides. The number of explanatory  
204 variables included in the final model had to be limited to avoid multicollinearity. Body mass index was  
205 included in the model, as it significantly differs between the PCOS and control women, and obesity is  
206 suggested to affect HRV.[4] Mean arterial pressure was selected because it combines information from both  
207 SBP and DBP, and FAI was included in the model because it is considered a good indicator of  
208 hyperandrogenemia in women with PCOS,[2] and hyperandrogenemia has been suggested to alter HRV in  
209 women with PCOS.[14] The homeostasis model assessment for insulin resistance was used as an estimate of  
210 insulin resistance, as it combines information from both fasting insulin and glucose levels, and triglycerides  
211 was included, as hypertriglyceridemia is a typical lipid abnormality in PCOS women and is linked to  
212 cardiovascular disease risks.[29] Anxiety was not included in the final multivariate model, because in the  
213 preliminary models using the stepwise method, it was always the first variable to be excluded. The results of  
214 linear regression models are reported as unstandardized coefficients (B), 95% confidence intervals for B,  $P$ -  
215 values, and  $R^2$  values for the model. The multicollinearity assumptions of the multivariate linear regression  
216 model were investigated using VIF, tolerance and eigenvalue indexes. In addition, a histogram of regression  
217 standardized residual frequency, normal P-P plot of regression-standardized residuals, and scatter plot figures

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3 218 were visually inspected to ensure that the model met the assumptions in the analysis. The data were analysed  
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5 219 using SPSS software (IBM SPSS Statistics 24.0, IBM Corp., New York, USA). A *P*-value < 0.05 was  
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7 220 considered statistically significant.  
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9 **221 Ethical approval**

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11 222 The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern  
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13 223 Ostrobothnia Hospital District approved the research. All the participants took part on a voluntary basis and  
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16 224 signed informed consent forms.  
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18 225 **Patient and public involvement statement**

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20 226 The patients, the public or any third parties were not involved in the design, conduct, reporting or dissemination  
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22 227 of our research.  
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## 228 RESULTS

### 229 Heart rate variability and baroreflex sensitivity

230 When compared with the control women, the women with PCOS in a seated position had a significantly higher  
231 mean HR and significantly lower values of rMSSD, LF<sub>RRi</sub>, HF<sub>RRi</sub> and baroreflex sensitivity (Figure 2).  
232 However, when adjusting for BMI at age 46, the women with PCOS had lower values only in rMSSD  
233 ( $P=0.033$ ) and HF<sub>RRi</sub> ( $P=0.016$ ) compared with the controls. In the standing position, the women with PCOS  
234 did not differ from the controls after adjustment for BMI at age 46 (data not shown).

### 235 Heart rate variability according to BMI group

236 After dividing the women according to BMI ( $<25$  or  $\geq 25$  kg/m<sup>2</sup>), the lean women with PCOS did not differ  
237 from the lean control women regarding the HRV parameters, whereas the overweight/obese women with PCOS  
238 had higher HRs and LF/HF ratios and lower rMSSD, LF<sub>RRi</sub> and HF<sub>RRi</sub> compared to the overweight/obese  
239 controls (Figure 3). It is noteworthy that the overweight/obese women with PCOS had higher BMIs and waist  
240 circumferences and had abnormal glucose metabolism and hyperandrogenemia more often than the  
241 overweight/obese control women (data not shown).

242 We also further divided the population into overweight (BMI 25–30 kg/m<sup>2</sup>) and obese (BMI>30  
243 kg/m<sup>2</sup>) groups. We found that the overweight women with PCOS ( $n=55$ ) had significantly lower rMSSD  
244 ( $2.54\pm 0.6$  vs.  $2.90\pm 0.6$ ,  $P=0.006$ ), LF<sub>RRi</sub> ( $4.91\pm 0.9$  vs.  $5.52\pm 0.9$ ,  $P=0.034$ ) and HF<sub>RRi</sub> ( $4.30\pm 1.2$  vs.  $5.00\pm 1.2$ ,  
245  $P=0.010$ ) compared to the overweight control women ( $n=328$ ), whereas HR ( $80.0\pm 11.2$  vs.  $75.8\pm 9.6$ ,  
246  $P=0.064$ ), LF/HF ratio ( $0.61\pm 0.9$  vs.  $0.51\pm 0.9$ ,  $P=0.187$ ), LF<sub>SBP</sub> ( $1.51\pm 0.8$  vs.  $1.80\pm 0.8$ ,  $P=0.087$ ), BRS  
247 ( $1.67\pm 0.4$  vs.  $1.81\pm 0.5$ ,  $p=0.182$ ) and  $\alpha$  ( $1.68\pm 0.4$  vs.  $1.81\pm 0.5$ ,  $P=0.182$ ) did not differ between these two  
248 groups. We found no significant differences between the obese PCOS and control women in any HRV  
249 parameters, but this might be due to a lack of statistical power, as our sample included only 23 obese women  
250 with PCOS (data not shown).

### 251 Linear regression analysis for rMSSD

252 The univariate linear regression analysis demonstrated that rMSSD was associated with BMI at ages 31 and  
253 46 and with waist circumference, anxiety, SBP, DBP, MAP and the serum levels of total cholesterol, HDL,  
254 LDL, triglycerides, glucose, insulin, HOMA-IR, SHBG, FAI and hsCRP at age 46 (Table 1). The body mass

index at age 14 was not associated with rMSSD. The multivariate linear regression analysis demonstrated that MAP, HOMA-IR and triglycerides were the strongest explanatory variables for rMSSD (Table 1).

**Table 1.** Univariate and multivariate linear regression models for heart rate variability measure (rMSSD) in women with PCOS

Variable	Univariate regression analysis				Multivariate Model* ( $B=4.075$ , $R^2=0.101$ )		
	B	95%CI for B	P-value	R <sup>2</sup>	B	95%CI for B	P-value
PCOS	NA	NA	NA	NA	-0.087	-0.190 – 0.016	0.099
BMI 14yr	-0.011	-0.022 – 0.000	0.057	0.002			
BMI 31yr	-0.022	-0.028 – -0.016	<0.001	0.022			
BMI 46yr	-0.028	-0.033 – -0.023	<0.001	0.055	-0.007	-0.016 – 0.002	0.140
Waist circumference	-0.012	-0.014 – -0.010	<0.001	0.061			
Systolic BP	-0.007	-0.009 – -0.005	<0.001	0.032			
Diastolic BP	-0.015	-0.017 – -0.013	<0.001	0.066			
Mean arterial pressure	-0.012	-0.014 – -0.010	<0.001	0.054	-0.007	-0.011 – -0.004	<0.001
Testosterone	0.042	-0.010 – 0.093	0.113	0.001			
SHBG	0.001	0.001 – 0.002	<0.001	0.005			
FAI	-0.121	-0.199 – -0.042	0.003	0.003	0.059	-0.055 – 0.173	0.309
Glucose	-0.121	-0.153 – -0.089	<0.001	0.023			
Insulin	-0.014	-0.018 – -0.011	<0.001	0.027			
HOMA-IR	-0.492	-0.585 – -0.399	<0.001	0.049	-0.256	-0.420 – -0.092	0.002
Total cholesterol	-0.067	-0.096 – -0.038	<0.001	0.009			
High-density lipoprotein	0.138	0.076 – 0.201	<0.001	0.008			
Low-density lipoprotein	-0.086	-0.114 – -0.058	<0.001	0.015			
Triglycerides	-0.243	-0.285 – -0.200	<0.001	0.050	-0.341	-0.558 – -0.123	0.002
High sensitive CRP	-0.071	-0.084 – -0.057	<0.001	0.034			
Anxiety (HSCL-25)	-0.195	-0.274 – -0.117	<0.001	0.010			

NA: not applicable. BMI: body mass index, BP: blood pressure, SHBG: sex hormone binding globulin, FAI: free androgen index, HOMA-IR: homeostasis model assessment–insulin resistance, CRP: C-reactive protein.

\* The multivariate model included PCOS, BMI, mean arterial pressure, FAI, HOMA-IR and triglycerides as explanatory variables. B: unstandardized coefficient from linear regression analysis, 95% CI: 95% confidence interval.

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60**Hyperandrogenemia**

265 **Hyperandrogenemia**  
266 Total or calculated free T at age 46 did not associate with rMSSD in the linear regression analysis (data not  
267 shown). The FAI was negatively associated with rMSSD (B= -0.121, 95% CI: -0.199 – -0.042,  $P=0.003$ ), but  
268 lost its significance after an adjustment for BMI. Similarly, the serum level of SHBG was positively associated  
269 with rMSSD (B=0.001, 95% CI: 0.001 – 0.002,  $P<0.001$ ), but lost its significance after a BMI adjustment.

For peer review only

## DISCUSSION

To our knowledge, this is the first study to investigate the cardiac autonomic function of late reproductive age women with PCOS. We demonstrate here, in a large general population-based setup, that late reproductive aged women with PCOS display reduced HRV, indicating reduced parasympathetic activity. However, in the multivariate linear regression analysis, the reduced HRV was associated with elevated BP, insulin resistance and dyslipidemia, but not PCOS *per se*, demonstrating that metabolic abnormalities are likely to be the main cause for reduced HRV in women with PCOS.

Previous studies have reported that women with PCOS display impaired cardiac autonomic function with decreased parasympathetic and increased sympathetic activity.[10-12] In line with our findings, a study of 75 overweight women with PCOS and 75 age- and BMI-matched controls suggested that impaired HR recovery, a marker for decreased parasympathetic activity, was caused by excess weight and insulin resistance and not by PCOS *per se*. [30] Furthermore, a cross-sectional study of 31 PCOS cases recruited from outpatient clinics reported that women with PCOS showed significantly decreased vagal activity, but the role of confounding metabolic abnormalities was not assessed, even though the women with PCOS had significantly higher BMIs, waist-hip ratios, BP, and serum levels of T and glucose than the control women.[10] Our findings are also in line with previous studies conducted in the general population in which an association between BP, glucose metabolism, dyslipidemia and cardiac autonomic function was reported.[5, 31]

In the present study, the overweight/obese women with PCOS had more adverse changes in HRV parameters than the overweight/obese control women, whereas the lean PCOS and control groups had comparable HRV values. However, the difference between the overweight/obese PCOS and control groups may have reflected the higher mean BMIs and waist circumferences and the higher prevalence of abnormal glucose metabolism in the overweight/obese PCOS group than in the overweight/obese controls. Previous studies have reported conflicting results regarding the effect of BMI on autonomic function in PCOS. A study of 19 overweight/obese women with PCOS and 21 overweight/obese control women reported that the women with PCOS had elevated muscle sympathetic nervous activity (MSNA), whereas HRV parameters did not significantly differ between the groups.[13] However, it was reported that the non-obese (BMI<30kg/m<sup>2</sup>) control and PCOS groups did not have significantly different standard deviation of all RRis (SDNN), rMSSD or percentage of successive differences in RRi > 50 ms (pNN50), whereas the obese (BMI≥30 kg/m<sup>2</sup>) women

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3 298 with PCOS had significantly decreased SDNN and pNN50, but not rMSSD.[32] The findings of that study  
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5 299 also demonstrated that sympathetic skin responses, investigated by electromyography from the median or tibial  
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7 300 nerve, were altered in both the non-obese and obese PCOS groups. These conflicting results might be explained  
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9 301 by the fact that the effect of obesity in sympathetic activation might be regional, as obesity has been reported  
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11 302 to increase sympathetic activity in the kidneys and skeletal muscle vasculature but to reduce it in the heart.[33]

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14 303 We found that serum T or FAI at age 46 did not associate with HRV after BMI adjustment (nor  
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16 304 did FAI or T at age 31, data not shown). In line with our findings, two previous studies found no significant  
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18 305 association between T and LF [12] or hormonal profile and HR recovery.[11] By contrast, the total T level was  
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20 306 reported to be inversely associated with LF and LF/HF ratio in the women with PCOS during mental stress  
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22 307 testing.[34] However, in the present study, we used the golden standard, a liquid-tandem mass-spectrometry  
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24 308 assay for T measurement, whereas the previous studies have used immunoassays.[11, 34] Another study found  
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26 309 higher MSNA in the normal weight women with PCOS compared with the normal weight controls, and the  
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28 310 strongest explanatory factors for higher MSNA in the women with PCOS were total and free T and  
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30 311 cholesterol.[14] However, MSNA describes sympathetic activity, whereas HRV mainly describes  
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32 312 parasympathetic activity.

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35 313 Also, the phenotype of PCOS was suggested to influence cardiac autonomic function, as the  
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37 314 anovulatory women with PCOS showed lower HRV response in mental stress tests than the controls, whereas  
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39 315 the ovulatory PCOS women showed intermediate values.[34] However, in that study, the women with  
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41 316 anovulatory PCOS had a different metabolic profile than those with ovulatory PCOS and the control  
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43 317 groups.[34] Furthermore, previous studies have indicated that anxiety was associated with reduced HRV in the  
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45 318 general population.[8] In our analysis, anxiety had a weak association with rMSSD in the univariate linear  
46  
47 319 regression model, although a further analysis revealed that metabolic abnormalities played a more important  
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49 320 role in the reduction of vagal activity.

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52 321 A recent study addressed the interrelated effects of insulin resistance, hyperandrogenism,  
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54 322 chronic inflammation and sympathetic dysfunction (evaluated by MSNA) in 49 PCOS and 23 control women;  
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56 323 based on the findings, the authors concluded that sympathetic dysfunction and hyperandrogenism were  
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58 324 associated with PCOS and that chronic inflammation might be the mediating factor between sympathetic  
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60 325 function, hyperandrogenism and insulin resistance.[35]. However, in the present study, the surrogate marker

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3 326 of sympathetic activity,  $LF_{SBP}$ , did not significantly differ between the PCOS and control women, suggesting  
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5 327 that in our population, the women with PCOS would not have increased sympathetic activity. This needs to be  
6  
7 328 interpret with caution as  $LF_{SBP}$  does not directly measure sympathetic activity. The frequency width of 0.04–  
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9 329 0.15Hz used in the present study could be considered a limitation, as a frequency width of 0.075–0.15 Hz is  
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11 330 affected by sympathetic modulation.[36] However, the LF oscillation of BP usually has a central frequency at  
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13 331 ~0.1 Hz that considerably varies in relation to sympathetic effect, [37][38] supporting the use of a wider  
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15 332 spectral band for  $LF_{SBP}$ . Of note, in our data, the women with PCOS had significantly lower BRS, but the  
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17 333 significance disappeared after adjusting for BMI, indicating that PCOS *per se* does not affect BRS.  
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20 334 The strength of our study is that it includes by far the largest sample size of women with PCOS  
21  
22 335 and HRV measurements. The data also add to the literature by representing a community-based approach.  
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24 336 Moreover, we were able to adjust for many confounding factors and to study the effect of metabolic  
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26 337 abnormalities. Also, this is the first study to investigate women with PCOS at a late fertile age. The definition  
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28 338 of the PCOS population could be considered a limitation; however, we have previously shown that the  
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30 339 population does display the typical endocrine, metabolic and psychological profiles of PCOS.[21-23]  
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32 340 Moreover, a recent genome-wide meta-analysis reported that the genetic architecture does not differ based on  
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34 341 the diagnostic criteria used for PCOS (self-reported, NIH criteria or non-NIH Rotterdam criteria),[39] thus  
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36 342 supporting our approach. Our study population included only women with Caucasian ethnicity; consequently,  
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38 343 our results are best generalized to PCOS women with Caucasian ethnicity, as ethnicity is known to affect many  
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40 344 traits of PCOS. In addition, a longer HRV recording period would have been more favorable, but due to  
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42 345 logistical reasons and the large number of subjects to measure, we had to limit the time. However, the recording  
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44 346 period of 150 second used in the present study should be reliable.[15]  
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47 347 In conclusion, in this community-based data set, the women with PCOS displayed reduced vagal  
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49 348 activity at a late fertile age with a strong association with metabolic abnormalities. The fact is that impaired  
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51 349 cardiovascular autonomic function (i.e. increased sympathetic/decreased parasympathetic activity) reflects the  
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53 350 risk for cardiac morbidity; this is most likely also the case in PCOS. This underlines the importance of the  
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55 351 active screening and treatment of metabolic abnormalities in women with PCOS, as also suggested by the  
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57 352 recently published guidelines for PCOS.[40] Previous studies have reported that in overweight/obese women  
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59 353 with PCOS, the impaired cardiovascular autonomic function could be improved by a 10-week energy  
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354 restriction,[41] a 3-month aerobic exercise training program[42] or acupuncture.[43] Importantly, it has been  
355 shown that the autonomic disturbance can be reversed with weight reduction,[44] which should justify  
356 increasing resources and efforts targeting weight management.

357  
358 **A DATA STATEMENT:** The data are available on request to the NFBC1966 Data Sharing Committee.  
359 NFBC1966 data sharing policies and processes meet the requirement and expectations of the Northern  
360 Ostrobothnia Hospital district policy on sharing of data from population and patient cohorts. Further  
361 information can be found at <https://www.oulu.fi/nfbc/>.

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376 and revision; AK: study design, data collection, analyses and interpretation, manuscript drafting and revision;  
377 ESV: data interpretation, manuscript drafting and revision; MT: data collection and interpretation, manuscript  
378 revision; KP: laboratory analyses and manuscript revision; JST: data interpretation and manuscript revision;  
379 SF: data interpretation and manuscript revision; LMP: data interpretation, manuscript drafting and revision;  
380 and TTP: study design, data interpretation, manuscript drafting and revision.

**REFERENCES**

- 1 March WA, Moore VM, Willson KJ, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;25:544–51. doi: 10.1093/humrep/dep399.
- 2 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25. doi: 10.1016/j.fertnstert.2003.10.004
- 3 Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;333:853–61. doi: 10.1056/NEJM199509283331307
- 4 Lambert GW, Straznicky NE, Lambert EA, et al. Sympathetic nervous activation in obesity and the metabolic syndrome--causes, consequences and therapeutic implications. *Pharmacol Ther* 2010;126:159–72. doi:10.1016/j.pharmthera.2010.02.002.
- 5 Stuckey MI, Tulppo MP, Kiviniemi AM, et al. Heart rate variability and the metabolic syndrome: a systematic review of the literature. *Diabetes Metab Res Rev* 2014;30:784–93 doi:10.1002/dmrr.2555.
- 6 Kakoly NS, Moran LJ, Teede HJ, et al. Cardiometabolic risks in PCOS: a review of the current state of knowledge. *Expert Rev Endocrinol Metab* 2019;14:23–33. doi: 10.1080/17446651.2019.1556094.
- 7 Sgoifo A, Carnevali L, Alfonso Mde L, et al. Autonomic dysfunction and heart rate variability in depression. *Stress* 2015;18:343–352 doi:10.3109/10253890.2015.1045868.
- 8 Paniccia M, Paniccia D, Thomas S, et al. Clinical and non-clinical depression and anxiety in young people: A scoping review on heart rate variability. *Auton Neurosci* 2017;208:1–14 doi:10.1016/j.autneu.2017.08.008.
- 9 Wulsin LR, Horn PS, Perry JL, et al. Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality. *J Clin Endocrinol Metab* 2015;100:2443–2448 doi:10.1210/jc.2015-1748.
- 10 Saranya K, Pal GK, Habeebullah S, et al. Assessment of cardiovascular autonomic function in patients with polycystic ovary syndrome. *J Obstet Gynaecol Res* 2014;40:192–199 doi:10.1111/jog.12154.
- 11 Tekin G, Tekin A, Kiliçarslan EB, et al. Altered autonomic neural control of the cardiovascular system in patients with polycystic ovary syndrome. *Int J Cardiol* 2008;130:49–55 doi:10.1016/j.ijcard.2007.08.037.
- 12 Yildirim A, Aybar F, Kabakci G, et al. Heart rate variability in young women with polycystic ovary syndrome. *Ann Noninvasive Electrocardiol* 2006;11:306–312 doi:10.1111/j.1542-474X.2006.00122.x.



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2  
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59  
60

- 409 13 Lambert EA, Teede H, Sari CI, et al. Sympathetic activation and endothelial dysfunction in polycystic ovary  
410 syndrome are not explained by either obesity or insulin resistance. *Clin Endocrinol (Oxf)* 2015;83:812–819  
411 doi:10.1111/cen.12803.
- 412 14 Sverrisdóttir YB, Mogren T, Kataoka J, et al. Is polycystic ovary syndrome associated with high  
413 sympathetic nerve activity and size at birth? *Am J Physiol Endocrinol Metab* 2008;294:576–581  
414 doi:10.1152/ajpendo.00725.2007.
- 415 15 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force  
416 of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.  
417 *Circulation* 1996;93:1043–1065.
- 418 16 Brown ZA, Louwers YV, Fong SL, et al. The phenotype of polycystic ovary syndrome ameliorates with  
419 aging. *Fertil Steril* 2011;96:1259–1265 doi:10.1016/j.fertnstert.2011.09.002.
- 420 17 Ollila MM, Piltonen T, Puukka K, et al. Weight gain and dyslipidemia in early adulthood associate with  
421 polycystic ovary syndrome: prospective cohort study. *J Clin Endocrinol Metab* 2016;101:739–747 doi:  
422 10.1210/jc.2015-3543.
- 423 18 Ollila ME, Kaikkonen K, Järvelin M, et al. Self-reported polycystic ovary syndrome is associated with  
424 hypertension: a Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* 2019;104:1221–1231  
425 doi:10.1210/jc.2018-00570.
- 426 19 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications.  
427 Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*  
428 1998;15:539–553.
- 429 20 Ollila MM, West S, Keinänen-Kiukaanniemi S, et al. Overweight and obese but not normal weight women  
430 with PCOS are at increased risk of Type 2 diabetes mellitus—a prospective, population-based cohort study. *Hum*  
431 *Reprod* 2017;32:423–431. doi: 10.1093/humrep/dew329.
- 432 21 Karjula S, Morin-Papunen L, Auvinen J, et al. Psychological distress is more prevalent in fertile age and  
433 premenopausal women with PCOS symptoms: 15-year follow-up. *J Clin Endocrinol Metab* 2017;102:1861–  
434 1869 doi: 10.1210/jc.2016-3863.

1

2

- 3 435 22 Taponen S, Martikainen H, Järvelin MR, et al. Hormonal profile of women with self-reported symptoms of  
4 oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab*  
5 436 2003;88:141–147. doi: 10.1210/jc.2002-020982
- 6  
7 437  
8  
9 438 23 Taponen S, Ahonkallio S, Martikainen H, et al. Prevalence of polycystic ovaries in women with self-  
10 reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland birth cohort 1966 study. *Hum*  
11 439 *Reprod* 2004;19:1083–1088. doi: 10.1093/humrep/deh214.
- 12  
13 440  
14  
15 441 24 Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*  
16 442 2013;4:26. doi:10.3389/fphys.2013.00026.
- 17  
18 443 25 Furlan R, Porta A, Costa F, et al. Oscillatory patterns in sympathetic neural discharge and cardiovascular  
19 variables during orthostatic stimulus. *Circulation* 2000;101:886–92. doi:10.1161/01.cir.101.8.886.
- 20 444  
21  
22 445 26 Pagani M, Somers V, Furlan R, et al. Changes in autonomic regulation induced by physical training in mild  
23 hypertension. *Hypertension* 1988;12:600–610 doi:10.1161/01.hyp.12.6.600.
- 24 446  
25  
26 447 27 Julien C. The enigma of Mayer waves: Facts and models. *Cardiovasc Res* 2006;70:12–21  
27 doi:10.1016/j.cardiores.2005.11.008.
- 28 448  
29  
30 449 28 Veijola J, Jokelainen J, Läksy K, et al. The Hopkins Symptom Checklist-25 in screening DSM-III-R axis-  
31 I disorders. *Nord J Psychiatry* 2003;57:119–123 doi:10.1080/08039480310000941.
- 32 450  
33  
34 451 29 Fauser BC, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary  
35 syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil*  
36 *Steril* 2012;97:28–38.e25. doi: 10.1016/j.fertnstert.2011.09.024.
- 37 452  
38  
39 453 30 Giallauria F, Palomba S, Manguso F, et al. Abnormal heart rate recovery after maximal cardiopulmonary  
40 exercise stress testing in young overweight women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
41 454 2008;68:88–93 doi:10.1111/j.1365-2265.2007.03004.x.
- 42 455  
43  
44 456 31 Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability  
45 and cardiovascular disease risk factors. *Int J Cardiol* 2010;141:122–131 doi:10.1016/j.ijcard.2009.09.543.
- 46 457  
47  
48 458 32 Hashim ZH, Hamdan FB, Al-Salihi AR. Autonomic dysfunction in women with polycystic ovary syndrome.  
49 *Iran J Reprod Med* 2015;13:27–34.
- 50 459  
51  
52 460 33 Vaz M, Jennings G, Turner A, et al. Regional sympathetic nervous activity and oxygen consumption in  
53 obese normotensive human subjects. *Circulation* 1997;96:3423–3429 doi:10.1161/01.cir.96.10.3423.
- 54 461  
55  
56 462

- 1  
2  
3 463 34 Di Domenico K, Wiltgen D, Nickel FJ, et al. Cardiac autonomic modulation in polycystic ovary syndrome:  
4  
5 464 does the phenotype matter? *Fertil Steril* 2013;99:286–292 doi:10.1016/j.fertnstert.2012.08.049.  
6  
7 465 35 Shorakae S, Ranasinha S, Abell S, et al. Inter-related effects of insulin resistance, hyperandrogenism,  
8  
9 466 sympathetic dysfunction and chronic inflammation in PCOS. *Clin Endocrinol (Oxf)* 2018;89:628–633  
10  
11 467 doi:10.1111/cen.13808.  
12  
13 468 36 Stauss HM. Identification of blood pressure control mechanisms by power spectral analysis. *Clin Exp*  
14  
15 469 *Pharmacol Physiol* 2007;34:362–368 doi:10.1111/j.1440-1681.2007.04588.x.  
16  
17  
18 470 37 Malliani A, Pagani M, Lombardi F, et al. Cardiovascular neural regulation explored in the frequency  
19  
20 471 domain. *Circulation* 1991;84:482–492 doi:10.1161/01.cir.84.2.482.  
21  
22 472 38 Kiviniemi AM, Frances MF, Tiinanen S, et al.  $\alpha$ -Adrenergic effects on low-frequency oscillations in blood  
23  
24 473 pressure and R-R intervals during sympathetic activation. *Exp Physiol* 2011;96:718–735  
25  
26 474 doi:10.1113/expphysiol.2011.058768.  
27  
28 475 39 Day F, Karaderi T, Jones MR, et al. Large-scale genome-wide meta-analysis of polycystic ovary syndrome  
29  
30 476 suggests shared genetic architecture for different diagnosis criteria. *PLoS Genet* 2018;14:e1007813  
31  
32 477 doi:10.1371/journal.pgen.1007813.  
33  
34  
35 478 40 Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based  
36  
37 479 guideline for the assessment and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*  
38  
39 480 2018;89:251–268. doi: 10.1111/cen.13795.  
40  
41 481 41 Thomson RL, Buckley JD, Noakes M, et al. Heart rate recovery improves after weight loss in overweight  
42  
43 482 and obese women with polycystic ovary syndrome. *Fertil Steril* 2010;93:1173–1178  
44  
45 483 doi:10.1016/j.fertnstert.2008.12.003.  
46  
47 484 42 Giallauria F, Palomba S, Maresca L, et al. Exercise training improves autonomic function and inflammatory  
48  
49 485 pattern in women with polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf)* 2008;69:792–798  
50  
51 486 doi:10.1111/j.1365-2265.2008.03305.x.  
52  
53  
54 487 43 Stener-Victorin E, Jedel E, Janson PO, et al. Low-frequency electroacupuncture and physical exercise  
55  
56 488 decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am J Physiol Regul Integr*  
57  
58 489 *Comp Physiol* 2009;297:387–395. doi:10.1152/ajpregu.00197.2009.  
59  
60

1  
2  
3 490 44 Maser RE, Lenhard MJ. An overview of the effect of weight loss on cardiovascular autonomic function.  
4  
5 491 *Curr Diabetes Rev* 2007;3:204–211.  
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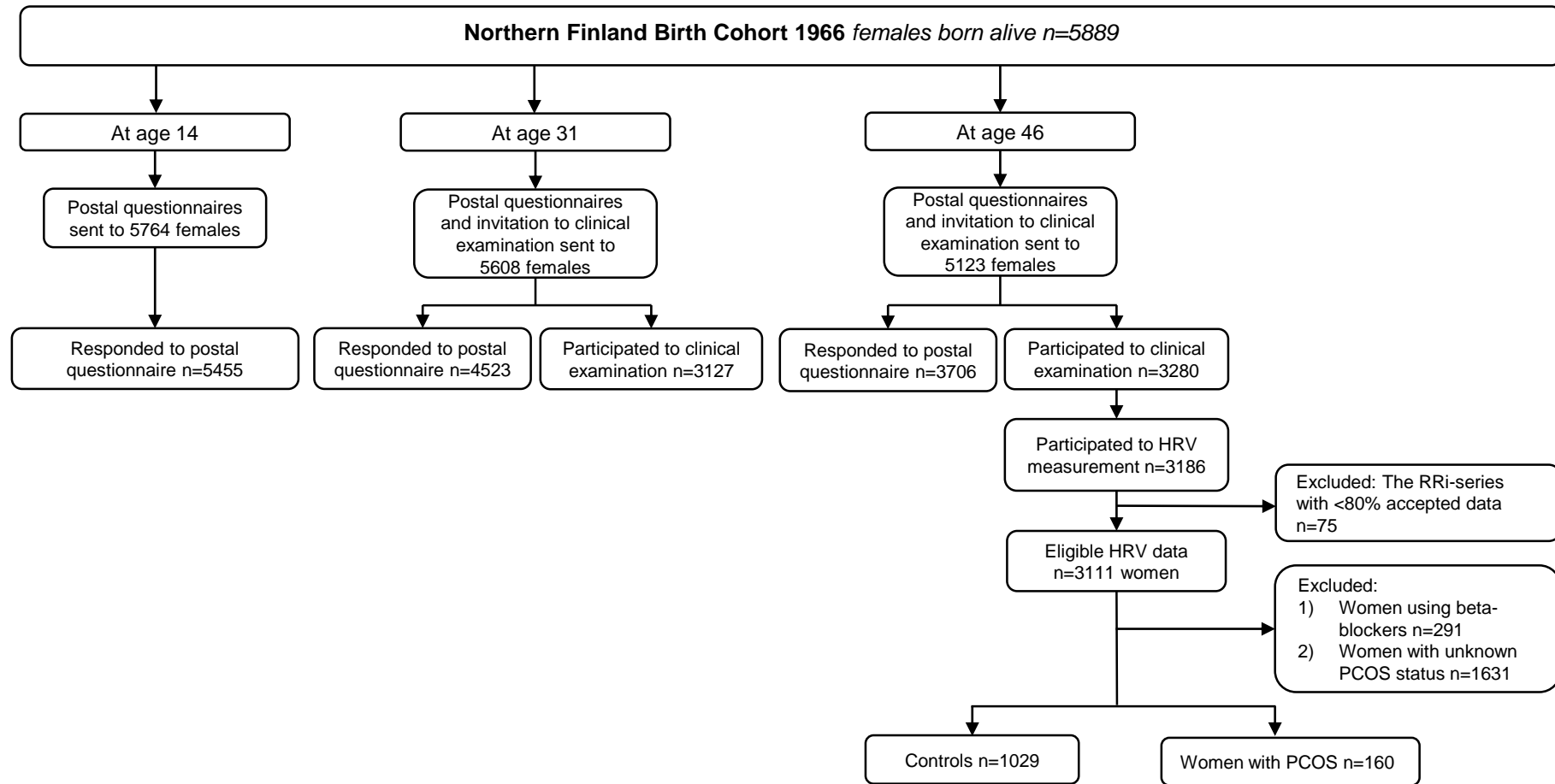
### 9 493 **FIGURE LEGENDS**

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11 494 **Figure 1.** Flow chart of the study. RRi: R-R interval.

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13 495 **Figure 2.** Heart rate variability parameters in controls and in women with PCOS at age 46 in seated position.

14  
15 496 The users of beta-blockers were excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles, and  
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17 497 the significance testing was made by Student's *t*-test (ln-transform was made to achieve normality). HR: heart  
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19 498 rate. rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals  
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21 499 (RRi), LF<sub>RRi</sub>: low frequency (0.04–0.15 Hz) power, HF<sub>RRi</sub>: high frequency (0.15–0.4 Hz) power,  $\alpha_1$ : short-term  
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23 500 fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex  
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25 501 sensitivity.  
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28 502 **Figure 3.** Heart rate variability parameters in controls and in women with PCOS at age 46 according to the  
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30 503 BMI group. The assessment of autonomic function in seated position. The users of beta blockers were  
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32 504 excluded. Values are mean  $\pm$  SD or median with 25% and 75% quartiles, and the significance testing was made  
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34 505 by Student's *t*-test (ln-transform was made to achieve normality). Statistically significant *P*-values are bolded.  
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36 506 rMSSD: The square root of the mean squared differences of successive normal-to-normal RR intervals (RRi),  
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38 507 LF<sub>RRi</sub>: low frequency (0.04-0.15 Hz) power, HF<sub>RRi</sub>: high frequency (0.15-0.4 Hz) power,  $\alpha_1$ : short-term fractal-  
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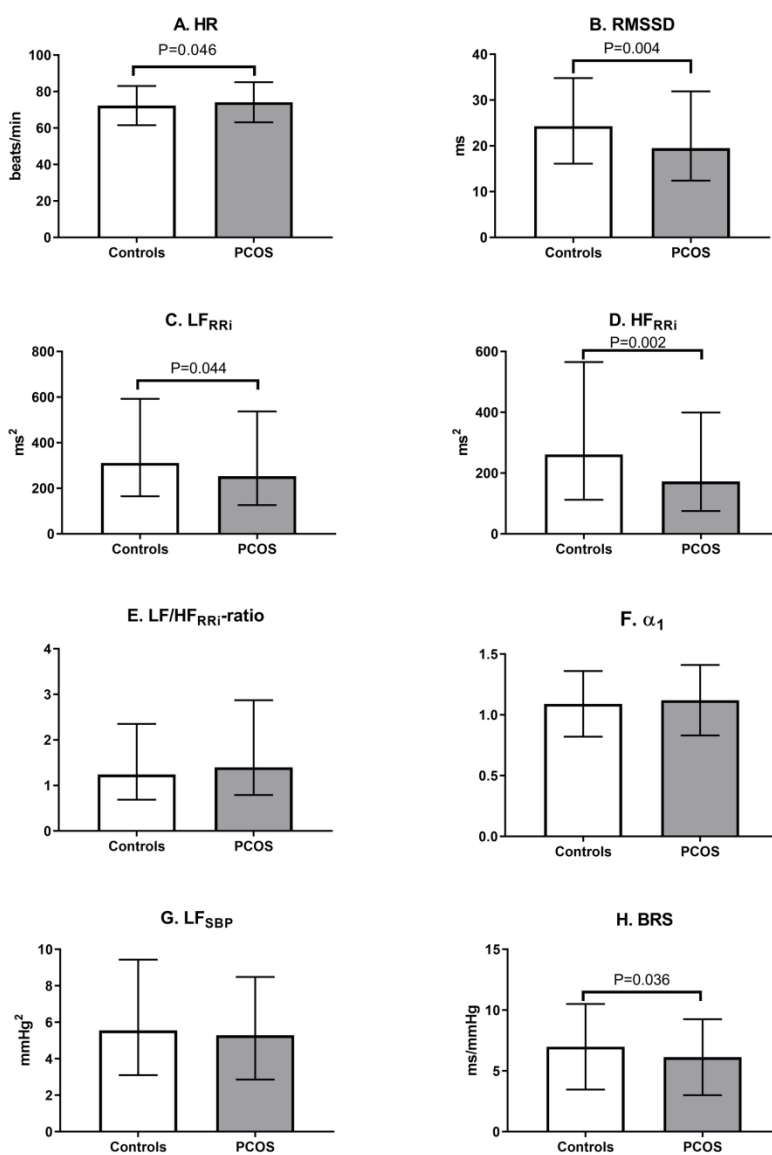


Figure 2

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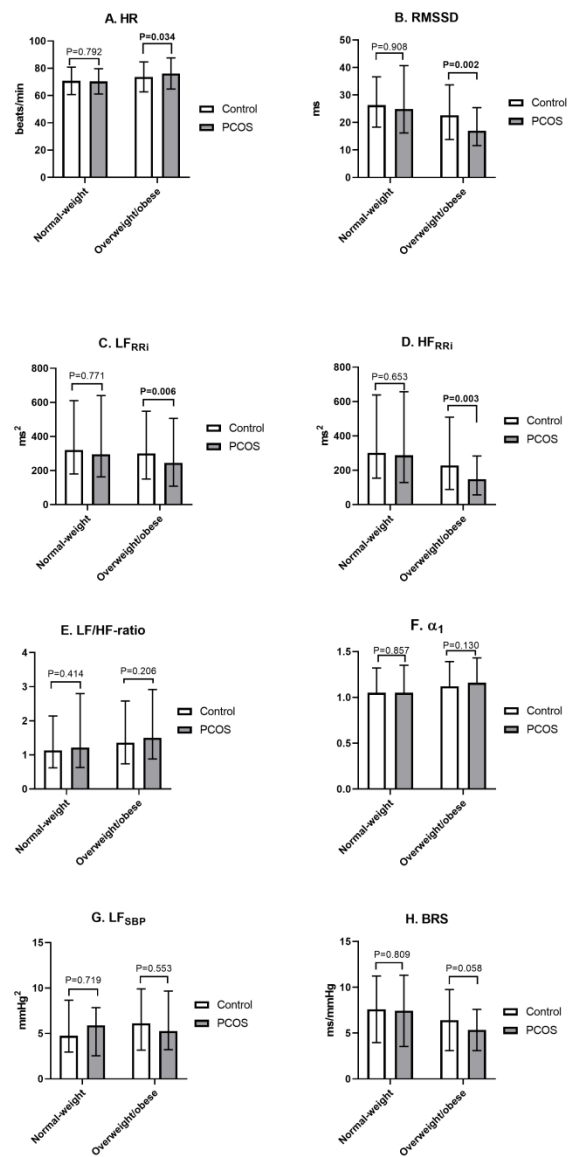


Figure 3

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## STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,2	
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4	
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4	
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	Page 5 and 6	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5 and 6	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	Page 5 and 6	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pages 6 – 8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 6 – 8	
Bias	9	Describe any efforts to address potential sources of bias	Page 15	
Study size	10	Explain how the study size was arrived at	Page 5 and 6	

Continued on next page



Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 8 and 9
		(b) Describe any methods used to examine subgroups and interactions	Page 8 and 9
		(c) Explain how missing data were addressed	NA
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	NA
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Pages 5 – 7
		(b) Give reasons for non-participation at each stage	Pages 5 – 7
		(c) Consider use of a flow diagram	Flow chart is included
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	Page 5
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Pages 9 – 12
		(b) Report category boundaries when continuous variables were categorized	Pages 9 – 10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	Pages 13–15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 13–15
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 15
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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