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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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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^2, *P*=0.002) and baroreflex sensitivity (6.13±3.12 *vs.* 6.99±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,

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

Keywords: PCOS, heart rate, heart rate variability, obesity, metabolism

Article Summary section

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Study population

The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large, prospective, general population-based, longitudinal birth cohort. All individuals with expected birth during 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began at the 24th gestational week, and the women were followed through age 46. The follow-up protocol of the cohort was previously described in detail, and the main data collection points during adulthood were carried out at ages 31 and 46 years.[16] Briefly, postal questionnaires were sent to all living cohort members with known addresses at ages 31 (81% answered, *n*=4,523/5,608) and 46 (72% answered, *n*=3,706/5,123) to collect information about health, behaviour, and social background. Postal questionnaires included an invitation to participate in clinical examinations at age 31 (77% participation rate, *n*=3,127/4,074), and at age 46 (64% participation rate, *n*=3,280/5,123). Weight and height were self-reported at age 14 (with the help of participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio of weight (kg) and height squared (m^2). Besides anthropometric measurements, clinical examinations at age

46 included blood sampling and assessment of cardiovascular health status, including measurement of systolic and diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound measurements, and evaluation of HRV and baroreflex sensitivity. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval after 15 minutes rest, and SBP and DBP averages were calculated.[17] The level of glucose metabolism was classified according to World Health Organization standards,[18] based on a 2-hour oral glucose tolerance test (performed at age 46) and a previously established diagnosis of type 2 diabetes.[19]

Definition of PCOS and control groups

At age 31, PCOS symptoms i.e. oligoamenorrhea and hirsutism were self-reported. Of all women who responded to questions regarding PCOS symptoms (*n*=4,523), after excluding pregnant women and those using hormonal preparations (*n*=1,459) or not permitting the use of their data (*n*=41), 4.1% (*n*=125) reported both oligoamenorrhea and hirsutism. The validity of this questionnaire to distinguish PCOS cases with typical hormonal, metabolic, and psychological traits characteristic to the syndrome, as well as ovarian morphology for PCOS, has been published previously.[19-22] At age 46, the postal questionnaire included a question on existing PCOS diagnosis, to which 181 subjects responded "yes." Consequently, women reporting both oligoamenorrhea and hirsutism and/or reporting PCOS diagnosis by age 46 were considered cases (*n*=279). Women without PCOS symptoms at age 31 and without diagnosis of PCOS by age 46 were considered controls (*n*=1,577). The characteristics of PCOS and control populations and the flow chart of the formation of the PCOS and control groups have been described previously.[16]

Evaluation of cardiac autonomic function

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

period before the beginning of the 3-minute recording period in the seated position. After that recording period, the participants stood up and remained still in a standing position for another three minutes while breathing normally.

Analysis of heart rate variability

The first 150 seconds of recording in a seated position and the last 150 seconds in a standing position were used in the analyses. The RRi data were edited based on visual inspection and the artefacts, and ectopic beats were removed and replaced by the local average (Hearts 1.2, University of Oulu, Oulu, Finland). Sequences with ≥10 consecutive beats of noise or ectopic beats were deleted. The RRi series with ≥ 80% accepted data were included in the analyses. The final study population included 1,029 controls and 160 women with PCOS. Mean heart rate, rMSSD (the square root of the mean squared differences of successive normal-to-normal RRi, spectral power densities (fast Fourier transformation, length 512 beats) including low frequency (LF: 0.04 – 0.15Hz, ms^2) and high frequency (HF: 0.15 – 0.40 Hz, ms^2) components of HRV and their ratio (LF/HF) were analysed.

Analysis of baroreflex sensitivity

Baroreflex sensitivity was assessed in participants who had the measures performed at the Oulu University Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals were imported to a custom-made stand-alone Matlab-based software (Biosignal Processing Team, University of Oulu, Oulu, Finland), where RRi and SBP values were extracted. Artefacts and ectopic beats were replaced using linear interpolation (<5% for accepted recording) and, thereafter, resampled at 2 Hz and detrended (<0.04 Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128 samples with 50% overlap) was performed to analyse low frequency (LF: 0.04-0.15 Hz) power of RRi and SBP oscillations for subsequent analysis of baroreflex sensitivity by the alpha method, if sufficient coherence (≥0.5) between LF oscillations in RRi and SBP was verified.

Laboratory methods

The laboratory methods have been previously described in detail.[16] At age 46, sex hormone binding globulin (SHBG) was assayed by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Llanberis, UK). The serum samples for testosterone (T) were assayed using Agilent triple quadrupole 6410 LC/MS equipment (Agilent Technologies, Wilmington, DE, USA). Free androgen index (FAI) was calculated using

the following equation: (100xT)/SHBG. Serum total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides were determined using an enzymatic assay method. Fasting plasma glucose (f-gluc) was analysed by an enzymatic dehydrogenase method (methods of cholesterol, HDL, LDL, triglycerides, and f-gluc: Advia 1800, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Fasting serum insulin (f-ins) was analysed by a chemiluminometric immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). High sensitivity C-reactive protein (hsCRP) was analysed by an immune-nephelometric assay (BN ProSpec, Siemens Healthcare Diagnostics Inc., Newark, DE, USA). The f-gluc and f-ins values were used to calculate the Homeostasis Model Assessment–insulin resistance (HOMA–IR) index (f-gluc x f-ins/22.5). The samples were analysed in NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189).

Hopkins Symptom Check List-25

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

Statistical methods

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

Univariate and multivariate linear regression analyses were used to study factors associated with HRV parameters. First, univariate linear regression models were used to reveal the parameters significantly associated with the outcome variable. Then, stepwise multivariate models were used to identify

the most important explanatory variables. The final multivariate model included the following variables as explanatory variables: PCOS, BMI at age 46, MAP, FAI, HOMA–IR, and triglycerides. The number of explanatory variables included in the final model had to be limited to avoid multicollinearity. Body mass index was included in the model, as it significantly differs between PCOS and control women, and obesity is suggested to affect HRV.[4] Mean arterial pressure was selected because it combines information from both SBP and DBP, and FAI was included in the model because it is considered a good indicator of hyperandrogenemia in women with PCOS,[2] and hyperandrogenemia has been suggested to alter HRV in women with PCOS.[24] Homeostasis Model Assessment–insulin resistance was used as an estimate of insulin resistance, as it combines information from both fasting insulin and glucose levels, and triglycerides was included, as hypertriglyceridemia is a typical lipid abnormality in PCOS women and is linked to cardiovascular disease risks.[25] Anxiety was not included in the final multivariate model, because in the preliminary models using the stepwise method, it was always the first variable to be excluded. The results of linear regression models are reported as unstandardized coefficients (B), 95% confidence interval for B, *P*-value, and R^2 value for the model. The multicollinearity assumptions of the multivariate linear regression model were investigated using VIF, tolerance, and Eigenvalue indexes. In addition, a histogram of regression standardized residual frequency, normal P-P plot of regression-standardized residuals, and scatterplot figures were visually inspected to ensure that the model met the assumptions in the analysis. The data were analysed using SPSS software (IBM SPSS Statistics 24.0, IBM Corp., New York, USA). A *P*-value <0.05 was considered statistically significant.

Ethical approval

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

Patient and Public Involvement statement

Patients, the public or any third parties were not involved in the design, or conduct, or reporting, or dissemination of our research.

RESULTS

Heart rate variability and baroreflex sensitivity

When compared with control women, women with PCOS in a seated position had a significantly higher mean HR and significantly lower values of rMSSD, LF_{RRi}, HF_{RRi}, and baroreflex sensitivity (Figure 2). However, when adjusting for BMI at age 46, women with PCOS had lower values only in rMSSD (P=0.033) and HF_{RRi} (P=0.016) compared with controls. In the standing position, women with PCOS did not differ from 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 ≥25 kg/m^2), normal weight women with PCOS did not differ from normal weight control women regarding HRV parameters, whereas overweight/obese women with PCOS had higher HRs and LF/HF ratios and lower rMSSD, LF_{RRi}, and HF_{RRi} compared to overweight/obese controls (Figure 3). It is noteworthy that overweight/obese women with PCOS had higher BMIs and waist circumferences and had abnormal glucose metabolism and hyperandrogenemia more often than overweight/obese control women (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, SBP, DBP, MAP, serum levels of total cholesterol, HDL, LDL, triglycerides, fasting glucose, fasting insulin, HOMA-IR, SHBG, FAI, hsCRP, and anxiety at age 46 (Table 1). 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.

	Univariate regression analysis				Multivariate Model* *(B=4.075, R^2=0.101)*		
Variable	**B**	**95%CI for B**	**P-value**	**R^2**	**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.

Hyperandrogenemia

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

DISCUSSION

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

Previous studies have reported that women with PCOS display impaired cardiac autonomic function with increased sympathetic activity and decreased parasympathetic and overall cardiac modulation.[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.*[26] 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 women with PCOS had significantly higher BMIs, waist-hip ratios, BP, T, and fasting glucose levels than control women.[10] In line with these studies, we also found that women with PCOS displayed mainly reduced vagal activity at age 46.

In the present study overweight/obese women with PCOS had more adverse changes in HRV parameters than overweight/obese control women, whereas normal-weight PCOS and control groups had comparable HRV values. However, the difference between overweight/obese PCOS and control groups may have reflected the higher mean BMIs and waist circumferences as well as 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 women with PCOS had elevated multi-unit and single-unit muscle sympathetic nervous system activity, whereas HRV parameters did not significantly differ between the groups.[13] On the other hand, it was reported that non-obese (BMI<30kg/m^2) 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 obese (BMI≥30 kg/m^2) women with PCOS had significantly decreased SDNN and pNN50, but not rMSSD.[27]

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

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

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

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

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]

Importantly, it has been shown that by weight reduction, the autonomic disturbance can be reversed,[37] setting the goal for increasing resources and efforts targeting weight management in affected women.

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

DECLARATION OF INTEREST: None declared.

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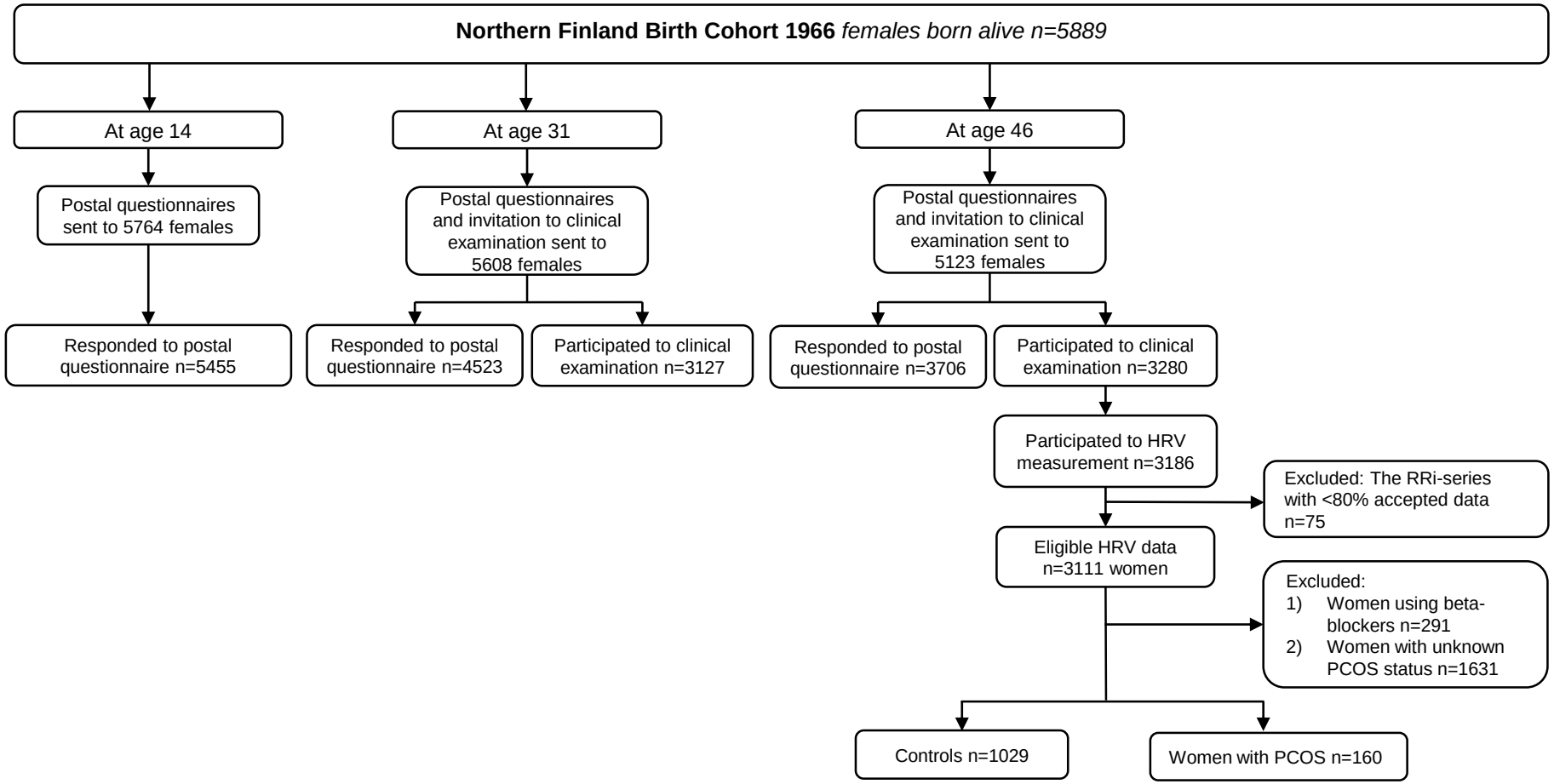
FIGURE LEGENDS

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 ± 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_{RRi}: low frequency (0.04-0.15 Hz) power, HF_{RRi}: high frequency (0.15-0.4 Hz) power, α_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 ± 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_{RRi}: low frequency (0.04-0.15 Hz) power, HF_{RRi}: high frequency (0.15-0.4 Hz) power, α_1: short-term fractal-

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


Northern Finland Birth Cohort 1966 females born alive n=5889
At age 14
At age 31
At age 46
Postal questionnaires sent to 5764 females
Postal questionnaires and invitation to clinical examination sent to 5608 females
Postal questionnaires and invitation to clinical examination sent to 5123 females
Responded to postal questionnaire n=5455
Responded to postal questionnaire n=4523
Participated to clinical examination n=3127
Responded to postal questionnaire n=3706
Participated to clinical examination n=3280
Participated to HRV measurement n=3186
Excluded: The RRi-series with <80% accepted data n=75
Eligible HRV data n=3111 women
Excluded:
1) Women using beta-blockers n=291
2) Women with unknown PCOS status n=1631
Controls n=1029
Women with PCOS n=160

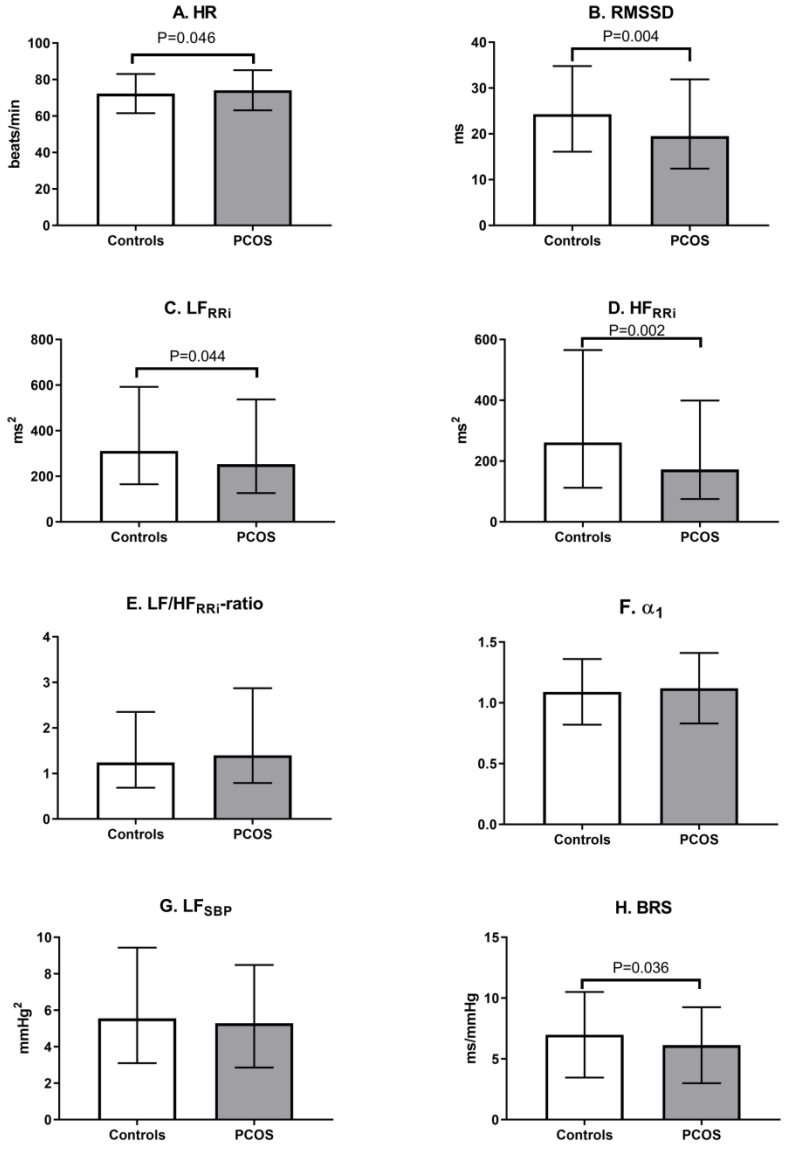


Figure 2

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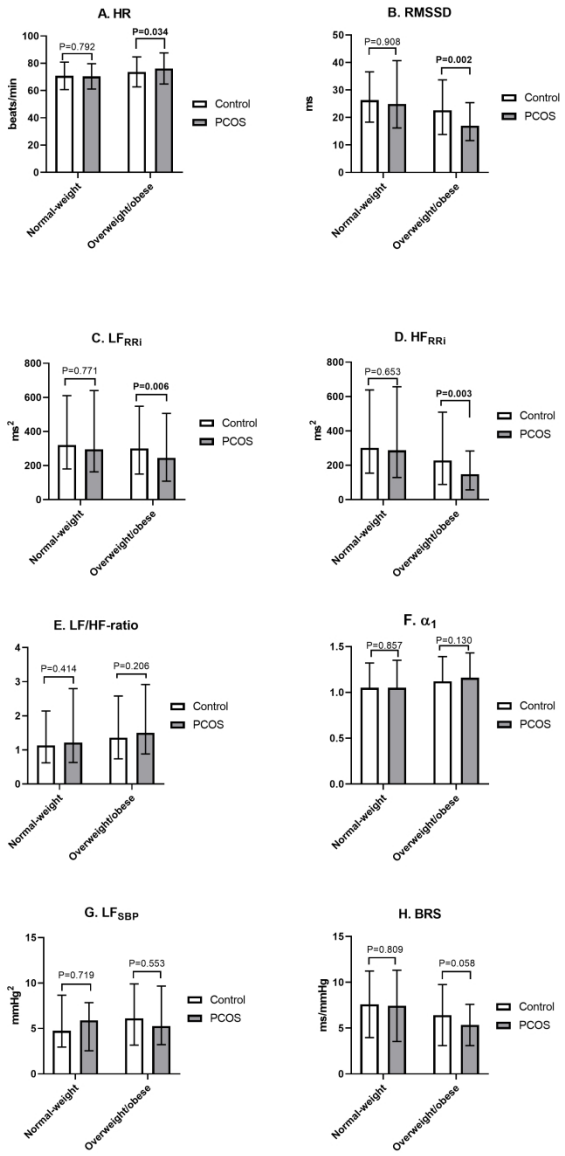

Figure 3

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STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
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	
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 5
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.

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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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Secondary Subject Heading:	Cardiovascular medicine, Reproductive medicine
Keywords:	PCOS, Heart rate, Heart rate variability, Obesity, Metabolism

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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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^2, *P*=0.002) and BRS (6.13±3.12 *vs.* 6.99±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

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

Keywords: PCOS, heart rate, heart rate variability, obesity, metabolism

Strengths and limitations of this study

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Study population

The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large, prospective, general population-based, longitudinal birth cohort. All individuals with expected births during 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began at the 24th gestational week, and the women were followed through age 46. The follow-up protocol of the cohort was previously described in detail, and the main data collection points during adulthood were carried out at ages 31 and 46 years.[17] Briefly, postal questionnaires were sent to all living cohort members with known addresses at ages 31 (81% answered, *n*=4,523/5,608) and 46 (72% answered, *n*=3,706/5,123) to collect information about health, behaviour and social background. Postal questionnaires included an invitation to participate in clinical examinations at age 31 (77% participation rate, *n*=3,127/4,074) and at age 46 (64% participation rate, *n*=3,280/5,123). Weight and height were self-reported at age 14 (with the help of the participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio of weight (kg) and height squared (m^2). Besides anthropometric measurements, the clinical examinations at

age 46 included blood sampling and assessments of cardiovascular health status, including systolic and diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound, and evaluations of HRV and baroreflex sensitivity. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval after 15 minutes rest by an automated, oscillometric BP device with an appropriately sized cuff (Omron Digital Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan), and SBP and DBP averages were calculated.[18] The level of glucose metabolism was classified according to World Health Organization standards,[19] based on a 2-hour oral glucose tolerance test (performed at age 46) and a previously established diagnosis of type 2 diabetes.[20]

Definition of PCOS and control groups

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

Evaluation of cardiac autonomic function

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

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

Analysis of heart rate variability

The first 150 seconds of recording in a seated position and the last 150 seconds in a standing position were used in the analyses. The RRi data were edited based on visual inspections, and the artefacts and ectopic beats were removed and replaced according to the local average (Hearts 1.2, University of Oulu, Oulu, Finland). Sequences with ≥10 consecutive beats of noise or ectopic beats were deleted. The RRi series with ≥ 80% accepted data were included in the analyses. The final study population included 1,029 controls and 160 women with PCOS. Mean heart rate, rMSSD (the square root of the mean squared differences of successive normal-to-normal RRi) and spectral power densities (fast Fourier transformation, length 512 beats), including low frequency (LF: 0.04–0.15Hz, ms^2) and high frequency (HF: 0.15–0.40 Hz, ms^2) components of HRV and their ratio (LF/HF), were analysed. Low frequency component reflects both the sympathetic and parasympathetic activity, whereas the HF component mainly describes parasympathetic activity. [15]

Analysis of baroreflex sensitivity

Baroreflex sensitivity (BRS) was assessed in the participants who had the measures performed at the Oulu University Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals were imported to custom-made, stand-alone Matlab-based software (Biosignal Processing Team, University of Oulu, Oulu, Finland), with which RRi and SBP values were extracted. Artefacts and ectopic beats were replaced using linear interpolation (< 5% for accepted recording) and, thereafter, resampled at 2 Hz and detrended (< 0.04 Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128 samples with 50% overlap) was performed to analyse the LF (0.04–0.15 Hz) power of RRi and SBP oscillations for subsequent analysis of BRS using the alpha method if sufficient coherence (≥0.5) between LF oscillations in RRi and SBP was verified. The present BRS method quantifies cardiac autonomic responses to spontaneous SBP variation, detected by baroreceptors in the aortic arch and the carotid sinus, which include both parasympathetic and sympathetic effects.[24] Concurrently, the LF oscillation of blood pressure (LF_{SBP},

0.04–0.15 Hz) was obtained and considered as a surrogate for peripheral sympathetic activity. However, the physiological background of LF_{SBP} is not fully established, as there are competing theories of central oscillation of sympathetic drive and BRS resonance.[25]

Laboratory methods

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

Hopkins Symptom Check List-25

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

Statistical methods

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

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

Univariate and multivariate linear regression analyses were used to study the factors associated with the HRV parameters. First, univariate linear regression models were used to reveal the parameters that were significantly associated with the outcome variable. Then, stepwise multivariate models were used to identify the most important explanatory variables. The final multivariate model included the following variables as explanatory variables: PCOS, BMI at age 46, MAP, FAI, HOMA–IR and triglycerides. The number of explanatory variables included in the final model had to be limited to avoid multicollinearity. Body mass index was included in the model, as it significantly differs between the PCOS and control women, and obesity is suggested to affect HRV.[4] Mean arterial pressure was selected because it combines information from both SBP and DBP, and FAI was included in the model because it is considered a good indicator of hyperandrogenemia in women with PCOS,[2] and hyperandrogenemia has been suggested to alter HRV in women with PCOS.[14] The homeostasis model assessment for insulin resistance was used as an estimate of insulin resistance, as it combines information from both fasting insulin and glucose levels, and triglycerides was included, as hypertriglyceridemia is a typical lipid abnormality in PCOS women and is linked to cardiovascular disease risks.[27] Anxiety was not included in the final multivariate model, because in the preliminary models using the stepwise method, it was always the first variable to be excluded. The results of linear regression models are reported as unstandardized coefficients (B), 95% confidence intervals for B, *P*-values, and R^2 values for the model. The multicollinearity assumptions of the multivariate linear regression model were investigated using VIF, tolerance and eigenvalue indexes. In addition, a histogram of regression standardized residual frequency, normal P-P plot of regression-standardized residuals, and scatter plot figures were visually inspected to ensure that the model met the assumptions in the analysis. The data were analysed using SPSS software (IBM SPSS Statistics 24.0, IBM Corp., New York, USA). A *P*-value < 0.05 was considered statistically significant.

Ethical approval

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

Patient and public involvement statement

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

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_{RRi}, HF_{RRi} 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_{RRi} (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 ≥25 kg/m^2), 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_{RRi} and HF_{RRi} 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^2) and obese (BMI>30 kg/m^2) groups. We found that the overweight women with PCOS (n=55) had significantly lower rMSSD (2.54±0.6 vs. 2.90±0.6, P=0.006), LF_{RRi} (4.91±0.9 vs. 5.52±0.9, P=0.034) and HF_{RRi} (4.30±1.2 vs. 5.00±1.2, P=0.010) compared to the overweight control women (n=328), whereas HR (80.0±11.2 vs. 75.8±9.6, P=0.064), LF/HF ratio (0.61±0.9 vs. 0.51±0.9, P=0.187), LF_{SBP} (1.51±0.8 vs. 1.80±0.8, P=0.087), BRS (1.67±0.4 vs. 1.81±0.5, p=0.182) and α (1.68±0.4 vs. 1.81±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

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

	Univariate regression analysis				Multivariate Model* *(B=4.075, R^2=0.101)*		
Variable	**B**	**95%CI for B**	**P-value**	**R^2**	**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.

Hyperandrogenemia

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

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.*[28] 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, 29]

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^2) 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^2) women

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

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

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

A recent study addressed the interrelated effects of insulin resistance, hyperandrogenism, chronic inflammation and sympathetic dysfunction (evaluated by MSNA) in 49 PCOS and 23 control women; based on the findings, the authors 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.[33]. However, in the present study, the surrogate marker

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

The strength of our study is that it includes by far the largest sample size of women with PCOS and HRV measurements. The data also add 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 a limitation; however, we have previously shown that the population does display the typical endocrine, metabolic and psychological profiles of PCOS.[21-23] 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),[37] 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 addition, a longer HRV recording period would have been more favorable, but due to logistical reasons and the large number of subjects to measure, we had to limit the time. However, the recording period of 150 second used in the present study should be reliable.[15]

In conclusion, in this community-based data set, the women with PCOS displayed reduced vagal activity at a late fertile age with a strong association with metabolic abnormalities. The fact is 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 the active screening and treatment of metabolic abnormalities in women with PCOS, as also suggested by the recently published guidelines for PCOS.[38] 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,[39] a 3-month aerobic exercise training program[40] or acupuncture.[41] Importantly, it has been shown that the autonomic disturbance can be reversed with weight reduction,[42] which should justify increasing resources and efforts targeting weight management.

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

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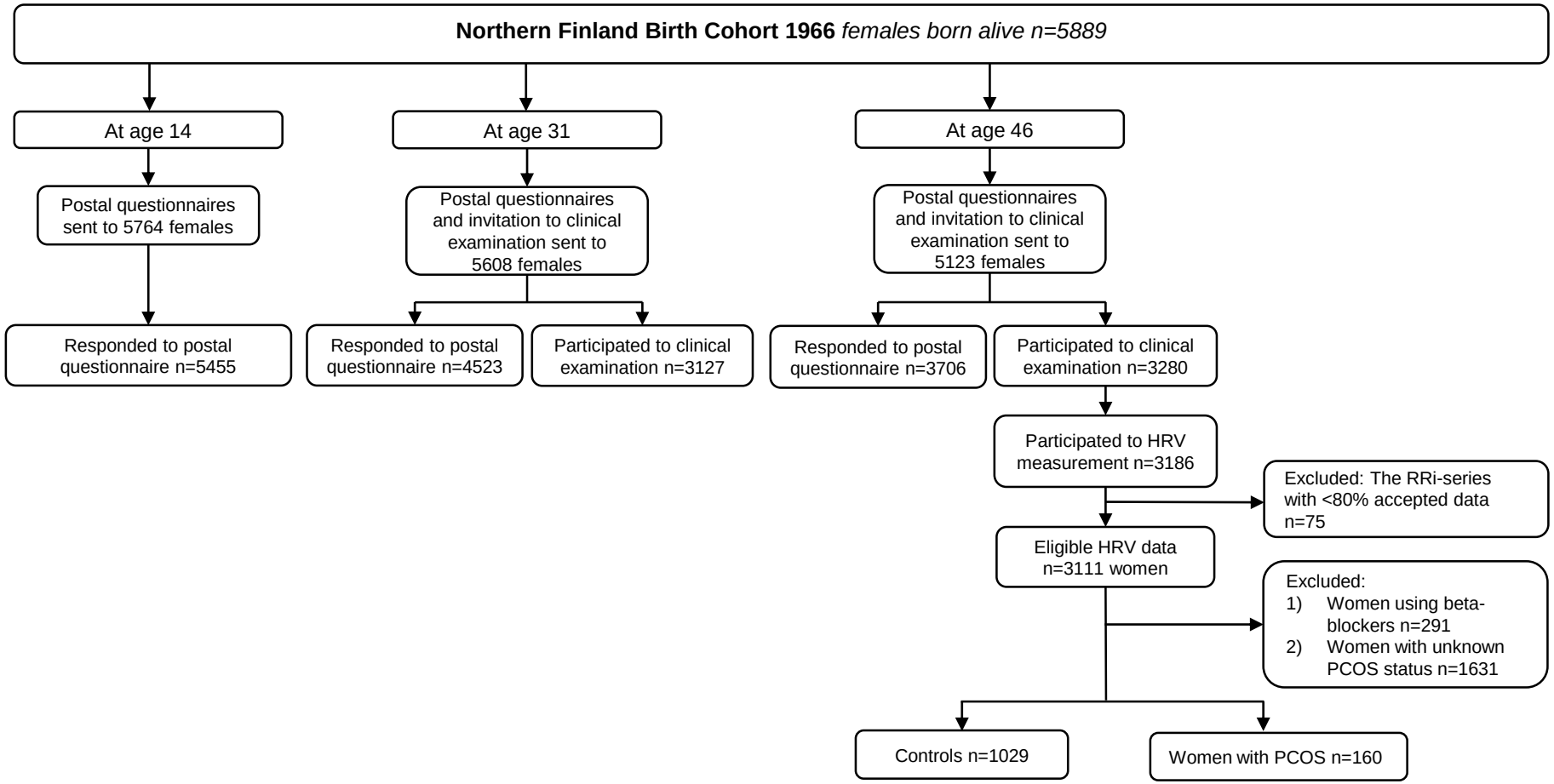
FIGURE LEGENDS

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 ± 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_{RRi}: low frequency (0.04–0.15 Hz) power, HF_{RRi}: high frequency (0.15–0.4 Hz) power, α_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 ± 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_{RRi}: low frequency (0.04-0.15 Hz) power, HF_{RRi}: high frequency (0.15-0.4 Hz) power, α_1: short-term fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex sensitivity.


Northern Finland Birth Cohort 1966 females born alive n=5889
At age 14
At age 31
At age 46
Postal questionnaires sent to 5764 females
Postal questionnaires and invitation to clinical examination sent to 5608 females
Postal questionnaires and invitation to clinical examination sent to 5123 females
Responded to postal questionnaire n=5455
Responded to postal questionnaire n=4523
Participated to clinical examination n=3127
Responded to postal questionnaire n=3706
Participated to clinical examination n=3280
Participated to HRV measurement n=3186
Excluded: The RRi-series with <80% accepted data n=75
Eligible HRV data n=3111 women
Excluded:
1) Women using beta-blockers n=291
2) Women with unknown PCOS status n=1631
Controls n=1029
Women with PCOS n=160

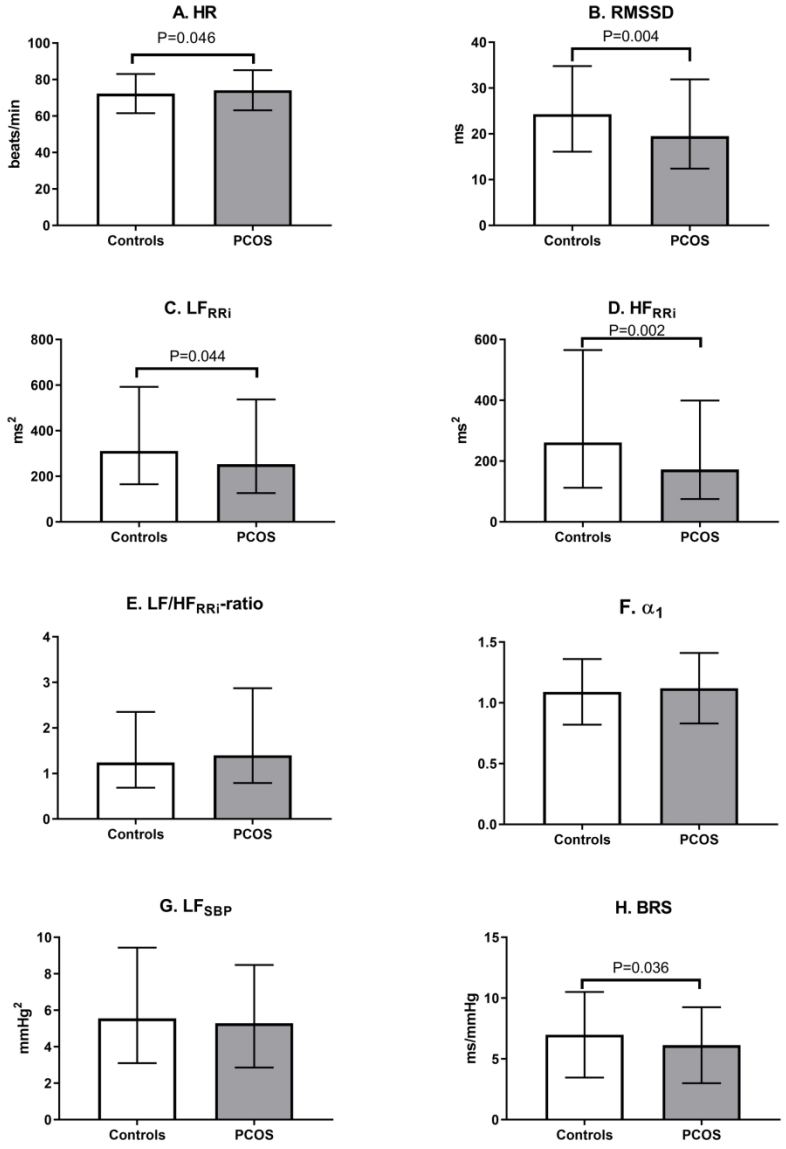


Figure 2

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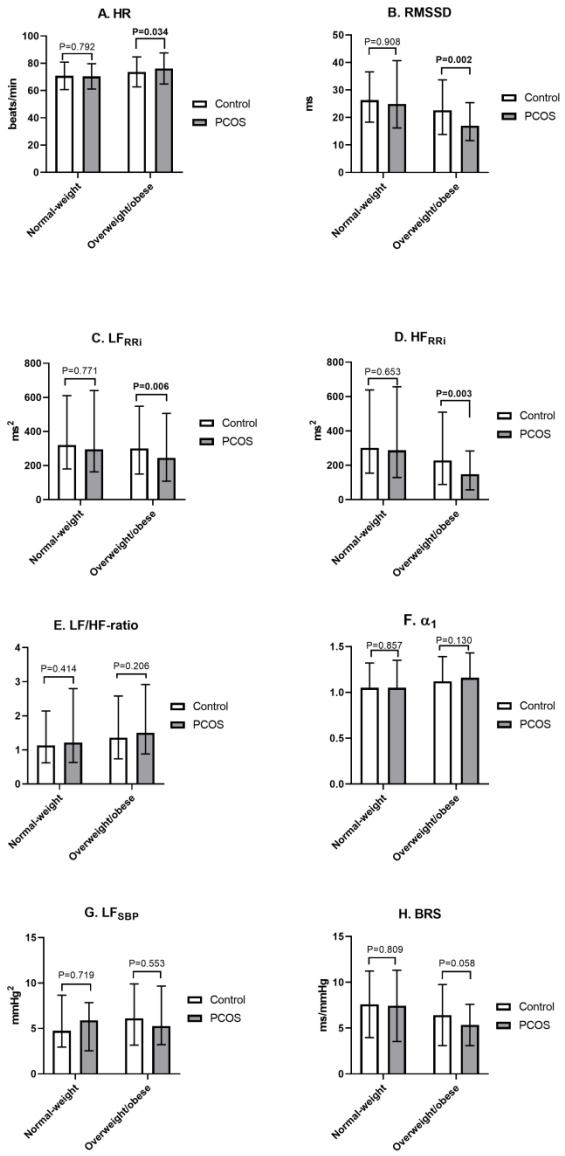

Figure 3

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STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
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	
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 5
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.

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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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Secondary Subject Heading:	Cardiovascular medicine, Reproductive medicine
Keywords:	PCOS, Heart rate, Heart rate variability, Obesity, Metabolism

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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Word count: 4009

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^2, *P*=0.002) and BRS (6.13±3.12 *vs.* 6.99±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

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

Keywords: PCOS, heart rate, heart rate variability, obesity, metabolism

Strengths and limitations of this study

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Study population

The study population comprises the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large, prospective, general population-based, longitudinal birth cohort. All individuals with expected births during 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included in this birth cohort (12,231 births, 5,889 females, 96.3% of all births during 1966 in that area). Enrolment in this database began at the 24th gestational week, and the women were followed through age 46. The follow-up protocol of the cohort was previously described in detail, and the main data collection points during adulthood were carried out at ages 31 and 46 years.[17] Briefly, postal questionnaires were sent to all living cohort members with known addresses at ages 31 (81% answered, *n*=4,523/5,608) and 46 (72% answered, *n*=3,706/5,123) to collect information about health, behaviour and social background. Postal questionnaires included an invitation to participate in clinical examinations at age 31 (77% participation rate, *n*=3,127/4,074) and at age 46 (64% participation rate, *n*=3,280/5,123). Weight and height were self-reported at age 14 (with the help of the participants' parents) and clinically measured at ages 31 and 46. Body mass index was calculated as the ratio of weight (kg) and height squared (m^2). Besides anthropometric measurements, the clinical examinations at

age 46 included blood sampling and assessments of cardiovascular health status, including systolic and diastolic blood pressure (SBP and DBP, respectively), carotid and cardiac ultrasound, and evaluations of HRV and baroreflex sensitivity. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. Brachial SBP and DBP were measured 3 times with a 1-minute interval after 15 minutes rest by an automated, oscillometric BP device with an appropriately sized cuff (Omron Digital Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan), and SBP and DBP averages were calculated.[18] The level of glucose metabolism was classified according to World Health Organization standards,[19] based on a 2-hour oral glucose tolerance test (performed at age 46) and a previously established diagnosis of type 2 diabetes.[20]

Definition of PCOS and control groups

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

Evaluation of cardiac autonomic function

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

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

Analysis of heart rate variability

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

Analysis of baroreflex sensitivity

Baroreflex sensitivity (BRS) was assessed in the participants who had the measures performed at the Oulu University Hospital (609 controls and 105 women with PCOS). Continuous ECG, BP, and respiration signals were imported to custom-made, stand-alone Matlab-based software (Biosignal Processing Team, University of Oulu, Oulu, Finland), with which RRi and SBP values were extracted. Artefacts and ectopic beats were replaced using linear interpolation (< 5% for accepted recording) and, thereafter, resampled at 2 Hz and detrended (< 0.04 Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128 samples with 50% overlap) was performed to analyse the LF (0.04–0.15 Hz) power of RRi and SBP

oscillations for subsequent analysis of BRS using the alpha method if sufficient coherence (≥0.5) between LF oscillations in RRi and SBP was verified. The present BRS method quantifies cardiac autonomic responses to spontaneous SBP variation, detected by baroreceptors in the aortic arch and the carotid sinus, which include both parasympathetic and sympathetic effects.[26] Concurrently, the LF oscillation of blood pressure (LF_{SBP}, 0.04–0.15 Hz) was obtained and considered as a surrogate for peripheral sympathetic activity. However, the physiological background of LF_{SBP} is not fully established, as there are competing theories of central oscillation of sympathetic drive and BRS resonance.[27]

Laboratory methods

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

Hopkins Symptom Check List-25

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

Statistical methods

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

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

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

Ethical approval

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

Patient and public involvement statement

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

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_{RRi}, HF_{RRi} 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_{RRi} (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 ≥25 kg/m^2), 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_{RRi} and HF_{RRi} 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^2) and obese (BMI>30 kg/m^2) groups. We found that the overweight women with PCOS (n=55) had significantly lower rMSSD (2.54±0.6 vs. 2.90±0.6, P=0.006), LF_{RRi} (4.91±0.9 vs. 5.52±0.9, P=0.034) and HF_{RRi} (4.30±1.2 vs. 5.00±1.2, P=0.010) compared to the overweight control women (n=328), whereas HR (80.0±11.2 vs. 75.8±9.6, P=0.064), LF/HF ratio (0.61±0.9 vs. 0.51±0.9, P=0.187), LF_{SBP} (1.51±0.8 vs. 1.80±0.8, P=0.087), BRS (1.67±0.4 vs. 1.81±0.5, p=0.182) and α (1.68±0.4 vs. 1.81±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

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

	Univariate regression analysis				Multivariate Model* *(B=4.075, R^2=0.101)*		
Variable	**B**	**95%CI for B**	**P-value**	**R^2**	**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.

Hyperandrogenemia

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

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^2) 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^2) women

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

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

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

A recent study addressed the interrelated effects of insulin resistance, hyperandrogenism, chronic inflammation and sympathetic dysfunction (evaluated by MSNA) in 49 PCOS and 23 control women; based on the findings, the authors 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.[35]. However, in the present study, the surrogate marker

of sympathetic activity, LF_{SBP}, did not significantly differ between the PCOS and control women, suggesting that in our population, the women with PCOS would not have increased sympathetic activity. This needs to be interpret with caution as LF_{SBP} does not directly measure sympathetic activity. The frequency width of 0.04–0.15Hz used in the present study could be considered a limitation, as a frequency width of 0.075–0.15 Hz is affected by sympathetic modulation.[36] However, the LF oscillation of BP usually has a central frequency at ~0.1 Hz that considerably varies in relation to sympathetic effect, [37][38] supporting the use of a wider spectral band for LF_{SBP}. Of note, in our data, the women with PCOS had significantly lower BRS, but the significance disappeared after adjusting for BMI, indicating that PCOS *per se* does not affect BRS.

The strength of our study is that it includes by far the largest sample size of women with PCOS and HRV measurements. The data also add 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 a limitation; however, we have previously shown that the population does display the typical endocrine, metabolic and psychological profiles of PCOS.[21-23] 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),[39] 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 addition, a longer HRV recording period would have been more favorable, but due to logistical reasons and the large number of subjects to measure, we had to limit the time. However, the recording period of 150 second used in the present study should be reliable.[15]

In conclusion, in this community-based data set, the women with PCOS displayed reduced vagal activity at a late fertile age with a strong association with metabolic abnormalities. The fact is 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 the active screening and treatment of metabolic abnormalities in women with PCOS, as also suggested by the recently published guidelines for PCOS.[40] 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,[41] a 3-month aerobic exercise training program[42] or acupuncture.[43] Importantly, it has been shown that the autonomic disturbance can be reversed with weight reduction,[44] which should justify increasing resources and efforts targeting weight management.

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

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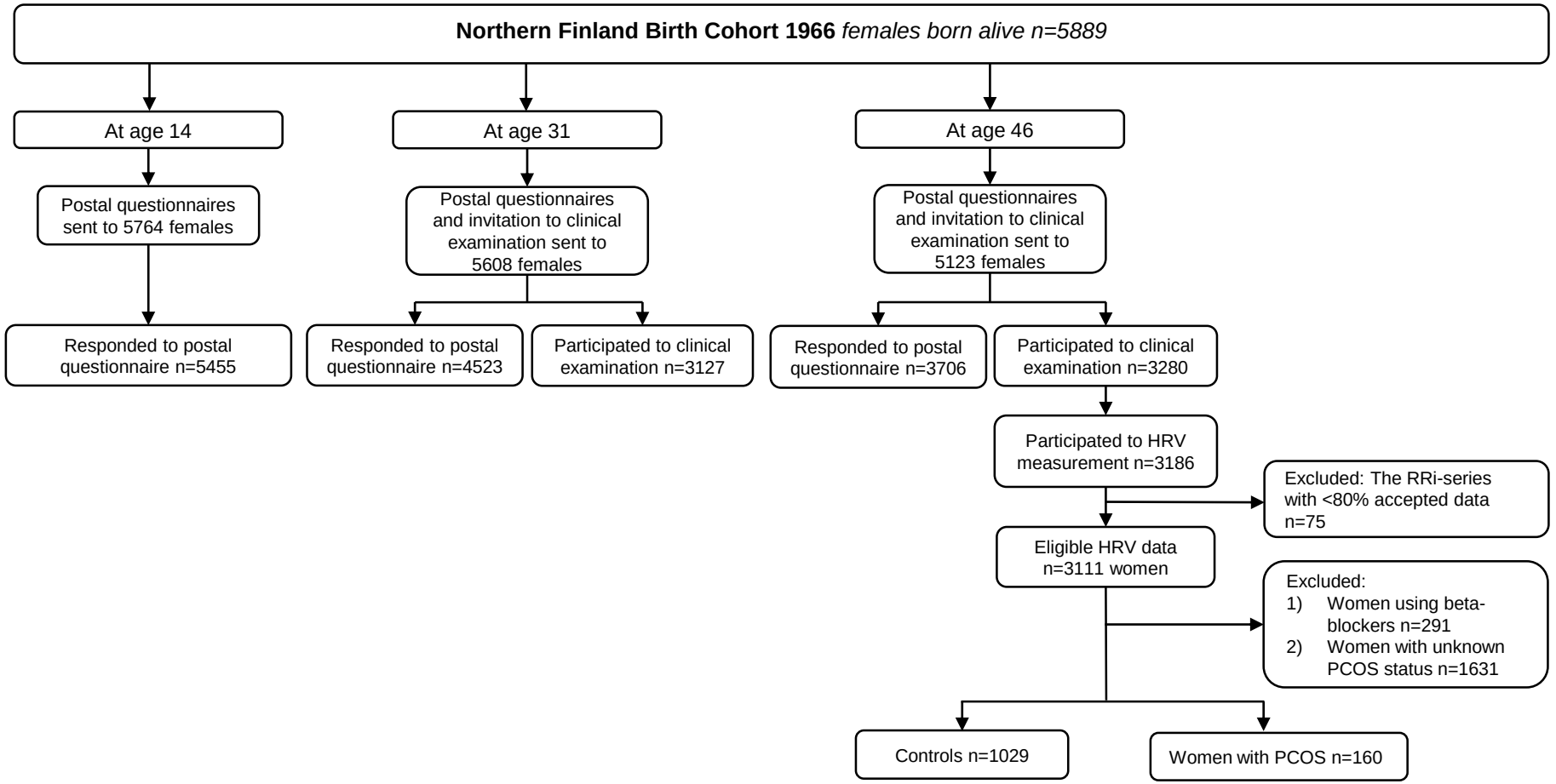
FIGURE LEGENDS

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 ± 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_{RRi}: low frequency (0.04–0.15 Hz) power, HF_{RRi}: high frequency (0.15–0.4 Hz) power, α_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 ± 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_{RRi}: low frequency (0.04-0.15 Hz) power, HF_{RRi}: high frequency (0.15-0.4 Hz) power, α_1: short-term fractal-like scaling exponent by detrended fluctuation analysis, SBP: systolic blood pressure, BRS: baroreflex sensitivity.


Northern Finland Birth Cohort 1966 females born alive n=5889
At age 14
At age 31
At age 46
Postal questionnaires sent to 5764 females
Postal questionnaires and invitation to clinical examination sent to 5608 females
Postal questionnaires and invitation to clinical examination sent to 5123 females
Responded to postal questionnaire n=5455
Responded to postal questionnaire n=4523
Participated to clinical examination n=3127
Responded to postal questionnaire n=3706
Participated to clinical examination n=3280
Participated to HRV measurement n=3186
Excluded: The RRi-series with <80% accepted data n=75
Eligible HRV data n=3111 women
Excluded:
1) Women using beta-blockers n=291
2) Women with unknown PCOS status n=1631
Controls n=1029
Women with PCOS n=160

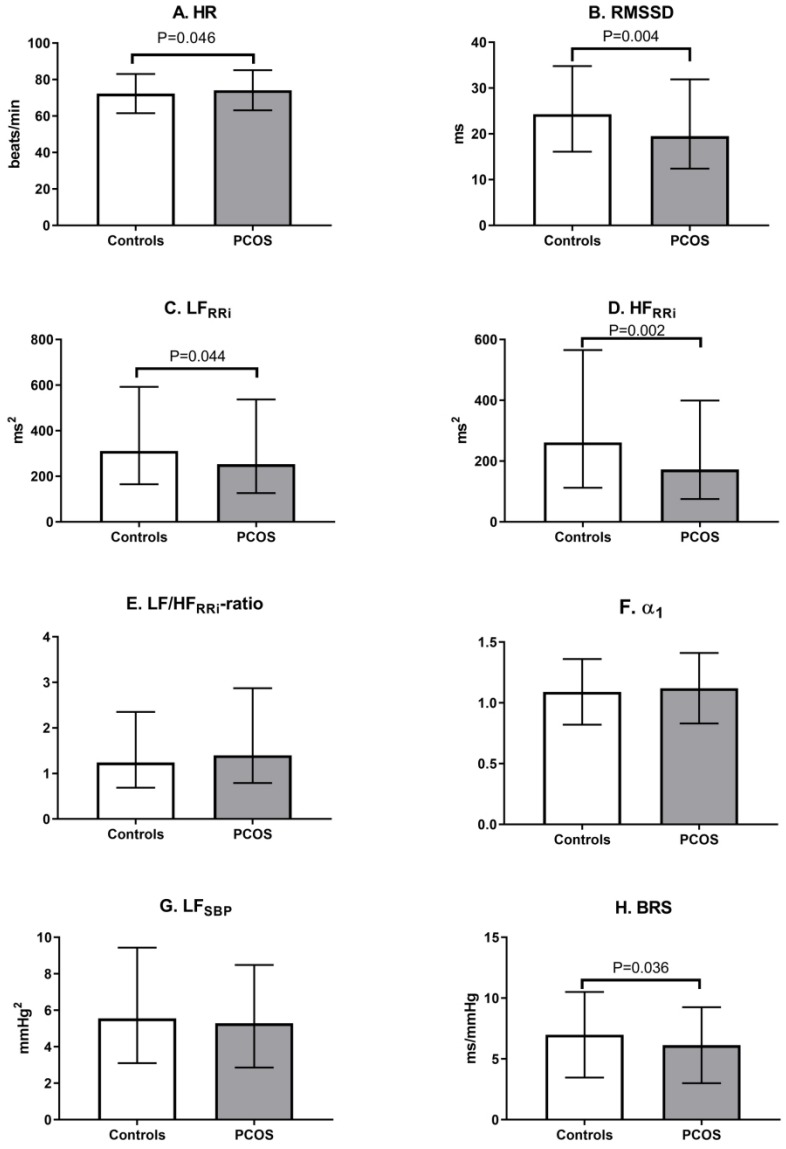


Figure 2

180x250mm (300 x 300 DPI)

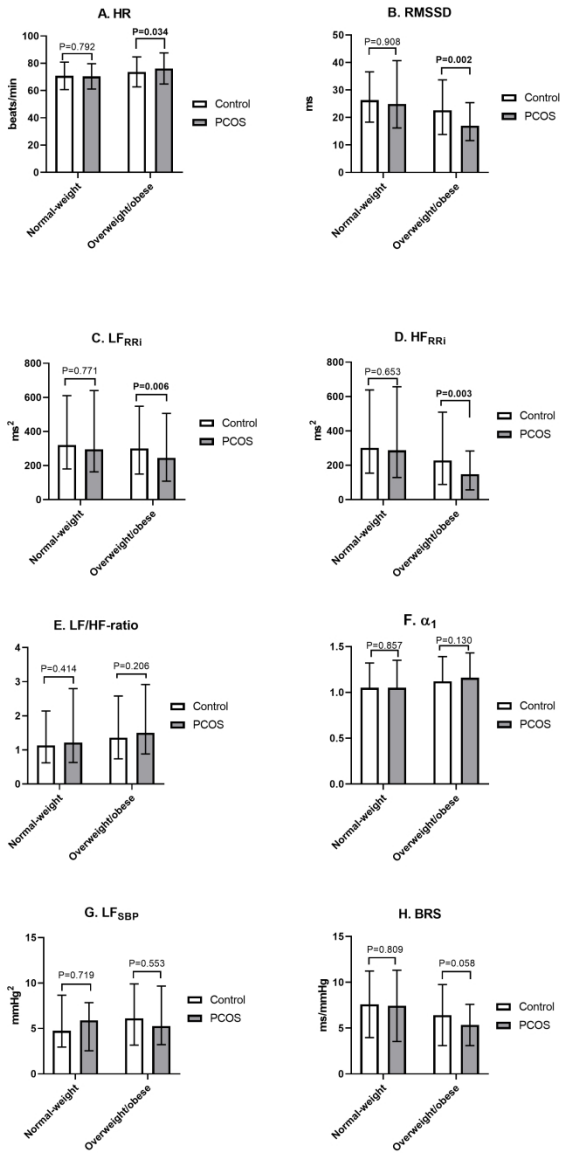


Figure 3

180x355mm (300 x 300 DPI)

STROBE Statement

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
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	
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5 and 6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 5
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.