CLINICAL RESEARCH ARTICLE

Self-reported Polycystic Ovary Syndrome is Associated with Hypertension: A Northern Finland Birth Cohort 1966 Study

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Precis: In a prospective cohort study, polycystic ovary syndrome was associated with hypertension, independently of body mass index, and increased cardiovascular morbidity, already in early premenopause.

Short title: PCOS and hypertension

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Abstract

Context: PCOS is associated with many traditional cardiovascular disease risk factors, but it is unclear whether PCOS is an independent risk factor for hypertension.

Objective: To investigate in a population-based set-up whether PCOS associates with the risk of hypertension independently of body-mass-index (BMI), and with cardiovascular manifestations.

Design: Cross-sectional assessments in the Northern Finland Birth Cohort 1966 at ages 31 and 46.

Setting: General community.

Participants: Women who reported both oligo/amenorrhea and hirsutism at age 31 and/or diagnosis of PCOS by age 46 (self-reported PCOS [srPCOS], n=279) and women without PCOS symptoms or diagnosis (n=1577).

Intervention: None.

Main Outcome Measures: Blood pressure (BP), BMI, cardiovascular manifestations.

Results: Use of antihypertensive medication was significantly more common in women with srPCOS. At age 31, women with srPCOS had significantly higher systolic (SBP) and diastolic BP (DBP) than control women (SBP: normal-weight: 119.9±13.2 *vs*. 116.9±11.4mmHg, P=0.017; overweight/obese: 126.1±14.3 *vs*. 123.0±11.9mmHg, P=0.031; and DBP: normal-weight: 75.5±10.0 *vs*. 72.4±9.6mmHg, P=0.003; overweight/obese: 80.7±11.8 *vs*. 78.0±10.6mmHg, P=0.031). At age 46, srPCOS was significantly associated with hypertension (AOR=1.56 [1.14–2.13]) independently of BMI, and with higher cardiovascular morbidity (6.8% vs. 3.4%, P=0.011). Hypertensive srPCOS displayed consistent, unfavorable changes in cardiac structure and function compared with controls.

Conclusion: Women with srPCOS displayed higher BP compared with controls already at early age and srPCOS was associated with hypertension independently of overweight/obesity. srPCOS was associated with increased cardiovascular morbidity in premenopausal women, suggesting that cardiovascular disease risk factors should be screened and efficiently managed early enough in women with PCOS.

Keywords: Polycystic ovary syndrome, body mass index (BMI), blood pressure (BP), cardiovascular morbidity

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting 6-18% of reproductive aged women (1,2). Even though the women with PCOS usually seek medical help for irregular menstruation, hirsutism or infertility, the syndrome is also characterized by several metabolic risk factors, such as insulin resistance, dyslipidemia, and metabolic syndrome (3). Most women with PCOS are overweight or obese (4) and they also more often present with increased abdominal fat accumulation than weight-matched controls (5). Insulin resistance and hyperandrogenism, together with excess weight, are key features of PCOS.

While PCOS is strongly associated with many traditional cardiovascular disease risk factors (3), it has remained unclear if PCOS is an independent risk factor for hypertension. An Australian study including 26 women with self-reported PCOS and hypertension (mean age: 28–33 years) reported that in normal-weight group, self-reported hypertension was more prevalent in PCOS than control group, whereas in the overweight/obese group the prevalence of hypertension was similar, and in the multivariate regression analysis PCOS was not significantly associated with hypertension (6). Furthermore, a hospital-based study population of 35 women with PCOS who had underwent wedge resection reported that these women had significantly higher prevalence of hypertension based on hospital discharge register information, even though clinically measured blood pressure (BP) levels were similar between PCOS and control groups (7). In contrast, several studies found comparable 24-hour ambulatory BP levels between PCOS and control groups (8-10). Moreover, the respective roles of body-mass-index (BMI) and PCOS as risk factors for hypertension are still controversial, as obesity has been reported to be the main determinant of hypertension in women with PCOS in some study (10), whereas others have claimed that hypertension was more prevalent in PCOS, independently of weight (6,11). Of note, previous studies have been limited with small number of study subjects (n=14–36) (6-10).

It is well known that increased BP may lead to altered cardiac structure and function and increase the risk of adverse cardiovascular outcome (12). Increased BP leads to a greater workload of the heart, inducing a compensatory cardiac muscle hypertrophy and increased left ventricle pressure, leading to enlargement of the left atrium (12). In a cross-sectional study on 18–30 years old women, women with PCOS displayed higher left atrium size and left ventricular mass index as well as lower left ventricular ejection

fraction (EF) and early- to late mitral flow velocity ratio but the role of hypertension was not assessed (13). However, it has been also reported that women with PCOS have similar cardiac structure and function as control women (14,15). Moreover, even though women with PCOS have an increased prevalence of cardiovascular disease risk factors and subclinical atherosclerosis, it is still unclear whether women with PCOS have an increased morbidity and mortality due to cardiovascular diseases (16).

The main goal of this study was to investigate the prevalence of hypertension and antihypertensive medication usage in a population-based set-up at ages 31 and 46 in women with self-reported PCOS (srPCOS) and to determine the respective roles of PCOS and obesity in affecting hypertension and associated echocardiographic examination findings at age 46. Secondly, we aimed to assess whether women with srPCOS have higher prevalence of cardiovascular morbidity compared with controls by age 46.

2. Materials and Methods

A. Study population

The study population arises from the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large prospective general population-based longitudinal birth cohort. All individuals with expected term during 1966 in the two northernmost provinces in Finland (Oulu and Lapland) were included into this birth cohort and the study population comprised all individuals born alive during that year (12,231 births, 5889 females, 96.3% of all births during 1966 in that area). Enrolment in this database begin at the 24th gestational week and, so far, this cohort population has been followed at birth, and at ages of 1, 14, 31 and 46. Postal questionnaires were sent at ages of 14, 31 and 46, and the present study utilized these data collection points. At ages of 31 and 46 clinical examinations were also performed (http://www.oulu.fi/nfbc/node/40683, last accessed June 9, 2018).

In 1980, at age 14, a postal questionnaire including questions about weight and height was sent to 5764 females and 95% of them (n=5455) responded with help from their parents. No other clinical data was collected at that age. In 1997, at age 31, a postal questionnaire, including questions about health, behavior, work and social background, was sent to 5608 women and 4523 (81%) of them responded. In addition, those living in Northern Finland or in the Helsinki metropolitan area (n=4074 women) were invited to a clinical examination. In total, 3127 (77%) women participated in a clinical examination including anthropometric measurements and blood samples for hormonal and metabolic parameters.

In 2012, at age 46, a comprehensive health research was carried out. A postal questionnaire and invitation to clinical examination were sent to all individuals alive and with known addresses (n=5123 women). Of them, 3706 (72%) women responded to the questionnaire and 3280 women (64%) participated to the clinical examination. Postal questionnaires covered all main health related issues, such as medication use, and all previously made disease diagnosis, life-style habits, education and work. Clinical examination included measurements of height, weight, waist and hip circumference, blood sampling and cardiovascular health status. A flow chart of the study is presented in Figure 1.

B. Definition of PCOS and control populations

At age 31, the postal questionnaire included questions on oligo/amenorrhoea: "Is your menstrual cycle often (more than twice a year) longer than 35 days?" and excessive body hair: "Do you have bothersome, excessive body hair growth?" Of the women who responded to these questions, 4.1% (n=125) reported both oligo/amenorrhoea and hirsutism (OA+H), after excluding pregnant women, women using hormonal preparations (n=1459) or not permitting the use of their data for data analysis (n=41). The validity of this questionnaire to distinguish PCOS cases has already been shown in our previous studies from the same cohort as the women with both OA+H present the typical hormonal, metabolic and psychological characteristics for the syndrome (4,17-19). At age 46, the postal questionnaire included the question: "Have you ever been diagnosed as having polycystic ovaries and/or polycystic ovary syndrome (PCOS)?" to which 181 responded "yes". Consequently, women who reported both OA+H at age 31 and/or diagnosis of PCO/PCOS by age 46 were considered as women with self-reported PCOS (srPCOS, n=279). Women without any PCOS symptoms at age 31 and without diagnosis of PCO/PCOS by age 46 were considered as controls (n=1577). More detailed information regarding this issue has been previously published (4).

C. Definitions of elevated BP and Hypertension

The assessment and diagnosis of hypertension were based on the European guidelines (20).

Measured elevated BP: In the clinical examination, brachial systolic and diastolic BP was measured twice at age 31 and three times at age 46 with one-minute interval after 15 minutes of rest on the right arm of the seated participants using a manual Mercury sphygmomanometer at age 31 and an automated, oscillometric blood pressure device with appropriately sized cuff (Omron Digital Automatic Blood Pressure Monitor Model M10-

IT) at age 46. Averages of systolic and diastolic BP were calculated, and elevated BP was defined as BP ≥130/85mmHg ("*measured elevated BP*" in the following text) at age 31 or 46. Women using antihypertensive medication were excluded from the analysis reporting mean values of systolic and diastolic BP.

Self-reported use of antihypertensive drugs: Information about antihypertensive medication was gathered from postal questionnaires at age 31 and 46, in which study subjects reported all their medication, dose and the reason for use. The use of antihypertensive drugs was defined as "*self-reported use of antihypertensive drugs*" at age 31 or 46.

Hypertension at age 46: In the following text "*hypertension at age 46*" (or *"hypertensive at age 46"*) is combined information from either self-reported use of antihypertensive medication at age 46 and/or measured elevated BP at age 46.

Self-reported diagnosis of hypertension at age 46: Last, we considered separately a group of women with self-reported diagnosis of hypertension in the questionnaire at age 46 ("self-reported diagnosis of hypertension at age 46").

D. Echocardiography

As a part of the clinical examinations at age 46, a subpopulation was enrolled to echocardiographic examinations in the Oulu research unit (Figure 1). As a rule, every second individual attending the clinical examination was randomly enrolled to echocardiographic examination without any preselection criteria, and without participant's own wish influencing the enrolment. All participants accepted to take part to the echocardiography. In total, echocardiography was performed in 645 women, including 37 women with srPCOS and 283 control women. The transthoracic 2D echocardiography was performed on-line by an experienced cardiologist (KK), using the General Electric Vivid E9 device with a M5S-D 1.5/4.6 MHz sector transducer for cardiovascular imaging (GE Health Medical, Horten, Norway). All measurements followed the American Society of Echocardiography guidelines (21). In addition, the global longitudinal strain was assessed off-line using the EchoPac 7 software (automated function imaging, AFI) (22). Global strain is a novel indicator of the overall systolic function of left ventricle, and may reveal heart disease already in early stage, when EF is still normal (23).

E. Definitions of cardiovascular disease manifestation and cardiovascular mortality

The ICD-8, ICD-9 and ICD-10 diagnostic codes for cardiovascular morbidity and events (I20, I21-25, I50, I60-I63, I63-I64, I65-I68, I69, I70, G45, G46) were identified from hospital discharge, hospital outpatient clinic and the basic health care registers, with data covering years 1972-2015, 1998-2015 and 2011-2015, respectively. In Finland, all individuals have a unique personal identification number. The individuals with serious acute illness are treated in public special health care centers, which report the diagnosis to the hospital discharge and hospital outpatient clinic registers. The data from national registers can be linked to an individual. Cardiovascular mortality data was retrieved from the mortality register of Statistics Finland, which covers the information about cause of death and time of death.

F. Statistical methods

BMI was calculated as the ratio of weight (kg) and height squared (m^2). BMI values at ages of 31 and 46 were obtained mainly from the clinical examination and supplemented with self-reported BMI if clinically measured BMI was not available. There were no differences between the self-reported or clinically measured BMI values. Women with BMI <25.0kg/m^2 were classified as normal weight, and with BMI ≥25.0kg/m^2 as overweight/obese (24), and this binary classification of BMI was used in the regression analysis. Differences in continuous parameters were analyzed by Student *t*-test and by Mann-Whitney *U*-test when appropriate. Continuous data were presented as means ± standard deviation (SD) or as median with [25% and 75% quartiles]. Categorical data were analyzed using cross-tabulation and Pearson's Chi-squared (χ^2) -test or Fisher's exact test. Binary logistic regression models were used to investigate the factors associated with hypertension and the results were reported as adjusted odds ratios (AORs) with [95% confidence intervals], with the adjustment for consumption of alcohol (not use, light, moderate, heavy use), smoking (never smoker, former smoker for > 6 months, former smoker for < 6 months and current smoker), education (basic, secondary, tertiary), and use of hormonal contraceptives (no, yes). Effect sizes were reported as Cohens *d*-values. Statistical analyses were performed using IBM SPSS Statistics 24.0 (SPSS, Inc., 1989, 2013, IBM Corp.). P-value <0.05 was considered statistically significant.

G. Ethical approval

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

3. Results

The clinical characteristics of srPCOS and control groups according to the BMI group are shown in Table 1. After BMI-adjustment by general linear modelling, the women with srPCOS did not significantly differ from controls regarding waist circumference, serum levels of sex hormone binding globulin, total cholesterol and low-density lipoprotein, and free-androgen index, whereas women with srPCOS had significantly higher serum testosterone and lower high-density lipoprotein concentration than control women (data not shown). The glucose metabolism parameters have already been previously reported (25).

A. Prevalence of self-reported diagnosis of hypertension and use of antihypertensive drugs

The prevalence of self-reported diagnosis of hypertension at age 46 was significantly greater among women with srPCOS compared with controls (Figure 2a, P<0.001) at age 46. Furthermore, self-reported use of antihypertensive medication was significantly more common among women with srPCOS compared with control women both at age 31 (Figure 2b, P=0.022) and 46 (Figure 2c, P<0.001).

B. Measured elevated BP at age 31 and 46

At age 31, normal-weight as well as overweight/obese women with srPCOS had significantly higher systolic and diastolic BP than normal-weight and overweight/obese control women, respectively. At age 46, in the normal-weight group, women with srPCOS displayed significantly higher diastolic, but not systolic BP compared with controls, whereas there were no significant differences between overweight/obese srPCOS women and overweight/obese controls (Table 1).

At age 46, women with srPCOS had significantly more often BP levels ≥ 140/90mmHg, than control women (32% [n=67/207] *vs.* 25% [n=332/1328], P=0.027). Of these 67 srPCOS women with BP≥ 140/90mmHg, 18 (27%) already used antihypertensive medication, whereas 46 (69%) did not. In three srPCOS cases, information about antihypertensive medication was lacking. If a cut-off of 130/80mmHg for

hypertension was used, as much as 71% (n=147/207) of women with srPCOS were hypertensive, compared to 59% (n=777/1328) of control women (P=0.001).

C. Echocardiography at age 46

The echocardiographic parameters did not significantly differ between srPCOS and control women. However, there was a small, non-significant trend towards higher measured values for interventricular septal thickness at diastole (IVSd), left ventricular mass index (LVMI) and left atrial systolic volume (LAESV), when compared to control women. There were no differences between srPCOS women and controls in diastolic filling pressure estimated with E/e' measurement (Table 2). The Cohens *d*-values varied from 0 to 0.33 representing very small or small effect sizes.

Hypertensive women with PCOS had significantly higher values for measured IVSd, LVMI and LAESV compared with normotensive controls. Estimated left ventricular filling pressure (E/e') was also increased in hypertensive PCOS women, compared with normotensive controls, suggesting modest alterations in diastolic function in this PCOS subgroup. Left ventricular systolic function was mildly decreased in hypertensive PCOS women (assessed by Global strain) even though the difference in LVEF was not statistically significant, when compared with other groups. The Cohens *d*-values varied from 0.33 to 0.82. Similarly, hypertensive control women exhibited significant unfavorable changes in the echocardiography parameters when compared to their normotensive counterparts, and the Cohens *d*-values varied from 0.46 to 0.62 (Table 3).

D. Binary logistic regression analysis

In the crude univariate binary logistic regression analysis, srPCOS was significantly associated with hypertension (Crude OR=1.99 [1.40–2.52]) at age 46 and this association remained significant (AOR=1.78 [1.32–2.40]) in the adjusted regression analysis (adjustment for consumption of alcohol, smoking, education, and use of combined contraceptive pills). In addition, in the regression analysis including also overweight/obesity at age 46 as covariate, srPCOS was associated with an increased risk of hypertension at age 46 by 1.5-fold (AOR=1.56 [1.14–2.13]), independently of overweight/obesity (AOR for overweight/obesity at age 46: 3.62 [2.92–4.49]).

E. Prevalence of cardiovascular disease manifestations by age 46

The prevalence of any cardiovascular disease was twice as high in women with srPCOS than in controls (6.8% vs. 3.4%, P=0.011) and the prevalence of myocardial infarction was already significantly higher in srPCOS women (1.8% vs. 0.5%, respectively, P=0.034) at this early premenopausal age (Table 4). By age 46, two women with srPCOS, but no woman in the control group, had died as a result of ischemic heart disease.

4. Discussion

To our knowledge, this is the largest general population-based prospective study with repeated cross-sectional assessments of Caucasian women with PCOS providing well-documented information about hypertension based on the use of antihypertensive medication, diagnosis of hypertension set by a physician and clinically measured BP level. Our main findings were that women with PCOS were significantly more often hypertensive and diagnosed with hypertension, and used more often antihypertensive medication compared with controls. Moreover, elevated BP was associated with unfavorable changes in echocardiographic parameters in hypertensive women with srPCOS compared with normotensive women. Last, premenopausal women with srPCOS exhibited significantly increased cardiovascular morbidity, even though these results need to be interpreted with caution, because of the small number of cases.

In the present study, women with srPCOS suffered more often from hypertension at age 46 and had significantly greater diastolic and systolic BP at age 31 than control women, regardless of BMI. In addition, at age 46, normal-weight women with srPCOS had significantly higher diastolic, but not systolic BP, when compared with normal-weight controls, whereas the difference between srPCOS and control women vanished in the overweight/obese groups. In line with the present findings, previous cross-sectional studies including adolescent (11) and young reproductive aged women (6) have reported significantly higher BP levels in normal-weight women with PCOS compared with normal-weight controls, whereas overweight and obese PCOS and control groups had similar prevalence of hypertension (6,11). These findings, in keeping with ours, suggest that PCOS has an independent effect on BP levels, even in young, normal-weight women. Of note, however, in the regression analysis, overweight/obesity was associated with hypertension by a greater odds ratio that for srPCOS, outlining the important role of excess weight as determinant of hypertension in women with PCOS. In addition, after including also the known potential effect modifiers/risk factors for elevated blood pressure (alcohol consumption, education, smoking, physical activity, diabetes, obstructive sleep apnea and

dyslipidemia) in the regression analysis, the results did not change (data not shown). In contrast, some studies found comparable 24-hour ambulatory BP levels between PCOS and control groups, but that finding might be due to small study populations (8-10). Of note, our study population included only women of Caucasian ethnicity and may therefore differ from other populations regarding the prevalence of hypertension and other cardiovascular risk factors, as race/ethnicity has also been reported to affect these risks in women with PCOS (26). Our results are therefore best generalized to Caucasian populations.

Women with srPCOS showed no statistically significant changes in echocardiographic features, although some non-significant, unfavorable trends were observed (increase in left atrial size, intraventricular septum thickness and left ventricular mass). These changes are recognized as adaptation of the heart to elevated levels of BP and are thought to predispose later to heart failure with preserved ejection fraction (HFpEF) (27). Hypertensive women with srPCOS had significantly higher values for left atrial size, intraventricular septum thickness and left ventricular mass, when compared to normotensive controls, whereas normotensive srPCOS and control groups had comparable cardiac structure and function, suggesting that elevated BP is an important determinant of the cardiac structure and function in women with PCOS. The relatively small number and young age of the women studied most likely explains why there were only few statistically significant differences in the echocardiographic parameters. The results of previous studies investigating cardiac structure and function in PCOS have been inconsistent. Two previous studies, investigating PCOS women in their 20s and 30s, reported significantly greater left atrial diameter and left ventricular mass in women with PCOS, independently of BMI, suggesting the early beginning of HFpEF or abnormalities in the filling phase of left ventricle (13,28). In contrast, other studies have not reported any significant difference in the echocardiographic parameters between PCOS and control women (14,15). The controversial findings of these previous studies might be explained by the differences in the age and ethnicity of the study groups as well as the criteria used for the diagnosis of PCOS.

In the present study, women with srPCOS had significantly higher prevalence of acute myocardial infarction by age 46 than control women, and the prevalence of any cardiovascular disease diagnosis was twice as high in the PCOS group, even though these were premenopausal women. However, these results need to be interpreted with caution, as the number of affected women with overt cardiovascular disease was very small. Likewise, no firm conclusions can be drawn as regards of the risk of CVD mortality

in the current study because the numbers were so small (two versus none). Previous studies have reported inconsistent findings with regards the risk of cardiovascular morbidity in women with PCOS, possibly because of variable study designs and ages of the participants. Recently, a Danish study reported that the event rate for CVD was significantly higher in premenopausal PCOS population compared to controls (29). Also, a nationwide, register-based study of PCOS women aged 30 years reported that PCOS was associated with a 2-fold higher risk of stroke and thrombosis, whereas the risk of other cardiovascular disease was not significantly increased (30). A 20-year, retrospective cohort study reported significantly increased odds ratio for myocardial infarction and significant correlation with age, history of hypertension and smoking with cardiovascular outcome in the PCOS patients (31). On the contrary, a 21-year follow-up study of 35 postmenopausal PCOS women with history of wedge resection, showed similar prevalence of myocardial infarction and stroke between PCOS and control group (7), and a retrospective observational study did not found any increased risk for large vessel disease in women with PCOS (mean age 30yr) (32). Additionally, a recent study reported that women with high postmenopausal androgen levels and retrospectively diagnosed with PCOS did not display an elevated risk for CVD (33). It has been postulated that later age at menopause and consequently, more prolonged oestrogen exposure, could alleviate the CVD burden in PCOS despite the high CVD risk profiles commonly present in these women. However, whether this is due to lack of reliable data for the diagnosis and/or the fact that the women in most studies have been too young for detecting CVD events, remains to be resolved in future, longitudinal studies.

The modest difference in BP levels observed in women with srPCOS is significant, as an increase of BP of 1.5–2 mmHg has been shown to have a marked impact on cardiovascular disease risk at population level (34). In that study, the reduction of even mildly and/or moderately elevated BP to normal levels was associated with a reduced risk of cardiovascular diseases (34). Of note, at age 46, one third of the women with srPCOS displayed measured BP levels over the generally accepted cut-off value for starting antihypertensive medication (BP≥140/90mmHg). Furthermore, of the srPCOS women with BP≥140/90 mmHg, 27% were hypertensive despite the use of antihypertensive medication and, even worse, over two thirds of them (69%) were probably not aware of their elevated BP levels. The follow-up evaluation of these women in the future will reveal whether the cardiovascular risk actually increases further in women with PCOS and clarify the respective roles of the risk factors linked to PCOS as regards cardiovascular morbidity.

The strengths of this study are the general population-based cohort study design, high participation and response rates, and low dropout rates. We have also previously reported that there were no significant differences between dropout and follow-up groups of women with OA+H at age 31 (4). Importantly, blood pressure was clinically measured at both ages 31 and 46. We were not able to adjust the results for some potential confounding factors (high sodium or low potassium intake, family history, stress and use of non-steroidal anti-inflammatory drugs), but adjustment for alcohol consumption, education, smoking, physical activity, diabetes, obstructive sleep apnea and dyslipidemia did not change the results. The cardiovascular event diagnoses were based on hospital discharge and outpatient registers, which have been shown to have a very high coverage and accuracy (88–98%) for vascular diseases in Finland (35). The main limitation of this study is that the definition of PCOS based on self-reported symptoms and diagnosis of PCOS, as that may be prone to misdiagnosis. However, we have previously shown that presence of both self-reported oligo-amenorrhea and hirsutism can accurately identify women with the typical endocrine, metabolic and psychological profiles of PCOS (17, 18,19), oligoamenorrhea being always self-reported data. Moreover, the presence of both oligo-amenorrhea and hirsutism fulfils both Rotterdam and NH criteria for PCOS. Despite this, our results suggest that even women with srPCOS, who probably have milder hormonal and metabolic alteration than PCOS women attending clinics, have increased risk for hypertension and cardiovascular diseases. Another limitation of the diagnosis is that the questionnaire at age 46 did not distinguish between those women with polycystic ovaries (PCO) and those with established PCOS. However, women with PCO were reported to show similar metabolic status as control women (36), and thus we would expect that the differences between the PCOS and control groups would have been even greater if we would have been able to exclude women with PCO only. Other limitations of this study include the relatively small number of women in the echocardiographic analysis and of those who had a diagnosis of cardiovascular disease.

In conclusion, hypertension and use of antihypertensive medication were significantly more common among women with srPCOS compared with controls, and srPCOS was significantly associated with hypertension, independently of overweight/obesity. Moreover, normal-weight women with srPCOS displayed significantly higher BP levels than normal-weight controls already in their 30s. The hypertensive srPCOS women showed a consistent trend towards unfavorable changes in the cardiac structure and function, exposing them later to increased risk of heart failure with preserved ejection fraction. In addition, an increased

prevalence of cardiovascular morbidity was already apparent in premenopausal women with srPCOS. Our results support strongly the recommendation of AE-PCOS Society about the ideal BP for women with PCOS to be 120 mmHg systolic and 80mmHg diastolic or lower (37). They are also in line with the results of the randomized, multicenter trial (SPRINT) (38), recommending a goal of systolic BP level less than 120 mm Hg for people at high cardiovascular risk. Last, the present findings strengthen further the need for early systematic screening and efficient treatment of hypertension in women with PCOS, as recommended by AE-PCOS Society (37) and the international evidence-based guideline for PCOS (39), as well as the importance of an active prevention and treatment of obesity in this particular group of women. Future prospective longitudinal studies are needed to establish the cause-effect relationship of PCOS and hypertension.

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7. Legends for Figures and Tables

Figure 1. The flow chart of Northern Finland Birth Cohort 1966.

Figure 2. Prevalence of self-reported diagnosis of hypertension at age 46 (a), self-reported use of antihypertensive medication at age 31 (b), and self-reported use of antihypertensive medication at age 46 (c). Data is analyzed using cross-tabulation and Chi-squared test. *** P-value < 0.001 and * P-value < 0.05.

Table 1. Clinical characteristics of control women and women with srPCOS according to their body-mass-indexes (BMIs) at age 31 and 46. Women were classified according to the BMI as to normal-weight women with BMI <25.00kg/m^2 and overweight/obese group with BMI ≥ 25.00kg/m^2. The number of women in each group is shown in parenthesis. Women using anti-hypertension drugs were excluded from the analysis reporting mean values of systolic and diastolic blood pressure (BP). The values are reported as mean ± standard deviation and differences between groups are analyzed using Student's *t*-test. P-value[1] is comparison between

normal-weight control and srPCOS women, and P-value[2] is comparison between overweight/obese control and srPCOS women.

Table 2. Echocardiography parameters in controls and in women with srPCOS. LV: left ventricle, EF: ejection fraction, IVSd: Interventricular septal end-diastole, LVIDd: Left ventricular internal diameter end-diastole, LAESV: left atrium end-systolic volume, LVMI: Left ventricle mass index.

Data are presented as mean± standard deviation. Differences between groups were analyzed by Student's *t*-test, as the data were normally distributed. Number on study subjects is shown in the parenthesis.

Table 3. Echocardiography parameters in controls and in women with srPCOS according to the presence of hypertension. BP: Blood pressure. LV: left ventricle, EF: ejection fraction, IVSd: Interventricular septal end-diastole, LVIDd: Left ventricular internal diameter end-diastole, LAESV: left atrium end-systolic volume, LVMI: Left ventricle mass index. Data are presented as mean ± standard deviation. Differences between groups were analyzed by Student's *t*-test, as the data were normally distributed. Number of study subjects in each group is shown in the parenthesis. Normotensive: Normal BP (systolic BP <130mmHg and diastolic BP <85mmHg) at age 31 and 46, and no antihypertensive medication at age 31 or 46. Hypertensive: Elevated BP (systolic BP ≥130 mmHg and/or diastolic BP ≥85 mmHg) at age 31 or 46 or antihypertensive medication at age 31 or 46.

P-value [1]: Comparison between normotensive srPCOS and control groups

P-value [2]: Comparison between hypertensive srPCOS and control groups

P-value [3]: Comparison between hypertensive srPCOS and normotensive control groups

P-value[4]: Comparison between hypertensive and normotensive control groups.

Table 4. Prevalence of cardiovascular morbidity by age 46 in controls and in women with srPCOS. The data is collected from the hospital discharge, the hospital outpatient clinic and the primary health care registers. The data was analyzed with cross-tabulation and Chi-square test and Fisher's Exact Test, when appropriate. Number of cases in each group is shown in parenthesis. * Combination of the ICD-8, ICD-9 and ICD-10 diagnostic codes for cardiovascular morbidity and events (angina pectoris, myocardial infarction, heart failure, atherosclerosis, transitory cerebral ischemia, intracranial hemorrhage, stroke, other disturbance in intracranial blood flow [I65-I68] and sequelae of cerebrovascular disease).