European Journal of Endocrinology, 2023, **189**, 96–105
https://doi.org/10.1093/ejendo/lvad077
Advance access publication 12 July 2023
Original Research

Women with PCOS have an increased risk for cardiovascular disease regardless of diagnostic criteria—a prospective population-based cohort study

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is associated with many cardiovascular disease (CVD) risk factors, such as obesity, type 2 diabetes mellitus and hypertension. However, it remains debatable whether the presence of multiple CVD risk factors translates to increased CVD events.

Design: A prospective, population-based Northern Finland Birth Cohort 1966.

Methods: Individuals with an expected date of birth in 1966 in Northern Finland have been followed from birth. Women in the cohort were classified as having PCOS according to either the National Institute of Health (NIH) criteria ($n = 144$) or the Rotterdam criteria ($n = 386$) at age 31, and they were compared to women without any PCOS features. The study population was re-examined at age 46, and the incidence of major adverse cardiovascular events (MACE), including myocardial infarction (MI), stroke, heart failure and cardiovascular mortality, was recorded up to age 53.

Results: During the 22-year follow-up, both women with NIH-PCOS and women with Rotterdam-PCOS had a significantly higher risk for cardiovascular events than control women. The BMI-adjusted hazard ratio (HR) for MACE in the Rotterdam-PCOS group and the NIH-PCOS group was 2.33 (1.26-4.30) and 2.47 (1.18-5.17), respectively. The cumulative hazard curves in both diagnostic categories began to diverge at age 35. Regarding the individual CVD endpoints, MI was significantly more prevalent in both women with NIH-PCOS ($P = .010$) and women with Rotterdam-PCOS ($P = .019$), when compared to control women.

Conclusions: PCOS should be considered a significant risk factor for CVD. Future follow-up will show how the risk of CVD events develops after menopausal age.

Keywords: PCOS, cardiovascular disease, epidemiology, diagnostic criteria, PCOS phenotype

Significance

This large, prospective, population-based cohort study was the first to demonstrate that women with PCOS, regardless of whether they were diagnosed according to the NIH or Rotterdam criteria, have a significantly increased risk for cardiovascular events. The risk of MACE already started to increase at age 35. This highlights the importance of actively screening and managing risk factors for CVD from early on.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of fertile-aged women, with a multifaceted and changing presentation throughout the life course. The community prevalence of PCOS has been reported to be as high as 18%.[1] The international guideline for the assessment and management of PCOS recommends that the syndrome is diagnosed according to modified Rotterdam criteria.[2] According to these criteria, the presence of two out of three features—oligoamenorrhea (OA), clinical or biochemical hyperandrogenism (HA), and/or polycystic ovarian morphology (PCOM) on ultrasonography—is sufficient to establish the diagnosis, after the exclusion of other diseases. The Rotterdam criteria produce four phenotypes, A–D (A: HA + OA + PCOM, B: HA + OA, C: HA + PCOM and D: OA + PCOM), which present with different hormonal and metabolic profiles, showing high cardiovascular disease (CVD) risk factor profile, especially in phenotype A but also among phenotypes A–C, representing the hyperandrogenic phenotype.[3] Even though the Rotterdam criteria have been suggested as the gold standard, the older National Institute of Health (NIH) 1990 criteria, including OA and HA, are still widely used.[4]

Received: January 19, 2023. **Revised:** May 23, 2023. **Editorial Decision:** May 31, 2023. **Accepted:** May 31, 2023

Women with PCOS have long-term exposure to traditional CVD risk factors as they often present with obesity, insulin resistance, abnormal glucose metabolism, dyslipidaemia, and elevated blood pressure (BP) as early as young adulthood or even adolescence.[5–8] Despite this, there are conflicting findings about whether the presence of multiple CVD risk factors translate to increased CVD events in women with PCOS.[5,9–14] These contradictory observations might be explained by limitations in the study design or the size of study populations, as well as variable definitions of PCOS, metabolic abnormalities, and cardiovascular events. Women with PCOS, diagnosed according to the NIH criteria, tend to display more metabolic abnormalities than women diagnosed according to the Rotterdam criteria,[15] which could also suggest that women diagnosed according to the NIH criteria could have a higher risk for CVD than women diagnosed according to the Rotterdam criteria. We also lack the knowledge of whether women with non-NIH PCOS phenotypes ie, phenotypes C and D, have an increased risk for CVD events.

Considering the debate about whether PCOS increases the risk for CVD events, we aimed to investigate this risk during a 22-year follow-up of a well-defined prospective population-based cohort and to further investigate whether the risk is increased irrespective of the diagnostic criteria used. We have previously shown that women in this cohort with self-reported PCOS had an increased prevalence of CVD events,[5] and we are now utilizing a longer follow-up period and a more detailed PCOS characterisation.

Methods

Study design and setting

This study was based on the prospective, general population-based Northern Finland Birth Cohort 1966 (NFBC1966) study. The cohort population includes all children born alive during 1966 (12 231 children, 5889 females) in the two northernmost provinces of Finland. The cohort population has been followed at four different time points: 1, 14, 31 and 46 years of age. Comprehensive questionnaires and clinical examinations were performed at ages 31 and 46; thus, the present study built on the two most recent studies. The detailed cohort description and follow-up protocol have been previously published.[16,17] Briefly, in 1997 (the 31-year follow-up), postal questionnaires regarding health, behaviour, work and social background were sent to all living individuals with known addresses ($n = 5608$ women), 81% ($n = 4523$) of whom responded. In addition, those living in northern Finland or in the Helsinki metropolitan area ($n = 4074$ women) were invited to a clinical examination, in which 77% ($n = 3127$) of the women participated. In 2012 (the 46-year follow-up), postal questionnaires and an invitation to the clinical examination were sent to all living individuals with known address ($n = 5123$ women). Of them, 72% ($n = 3706$) responded to the questionnaire and 64% ($n = 3280$ women) participated in the clinical examination. Figure 1 describes the NFBC1966 follow-up datapoints used in the present study.

Study population

Definition of PCOS groups

The formation of the PCOS groups has previously been described in detail.[18] Briefly, women with both OA and HA at age 31 were defined as NIH-PCOS ($n = 144$). Moreover, women who had at least two of the following three features—OA or HA, or anti-Müllerian hormone (AMH) ≥3.2 ng/mL (as a surrogate for ultrasonographic detection of PCOM)—were defined as Rotterdam-PCOS ($n = 386$). AMH was used to detect antral follicle excess in women who would otherwise have only one PCOS feature (OA or HA) when diagnosing PCOS according to the Rotterdam criteria. Although AMH has not yet been officially established as a part of PCOS diagnosis, this approach follows the upcoming 2023 PCOS Guideline (currently available for public consultant at https://whirlcre.edu.au/new-knowledge/pcos/guideline-public-consultation/, accessed April 16,

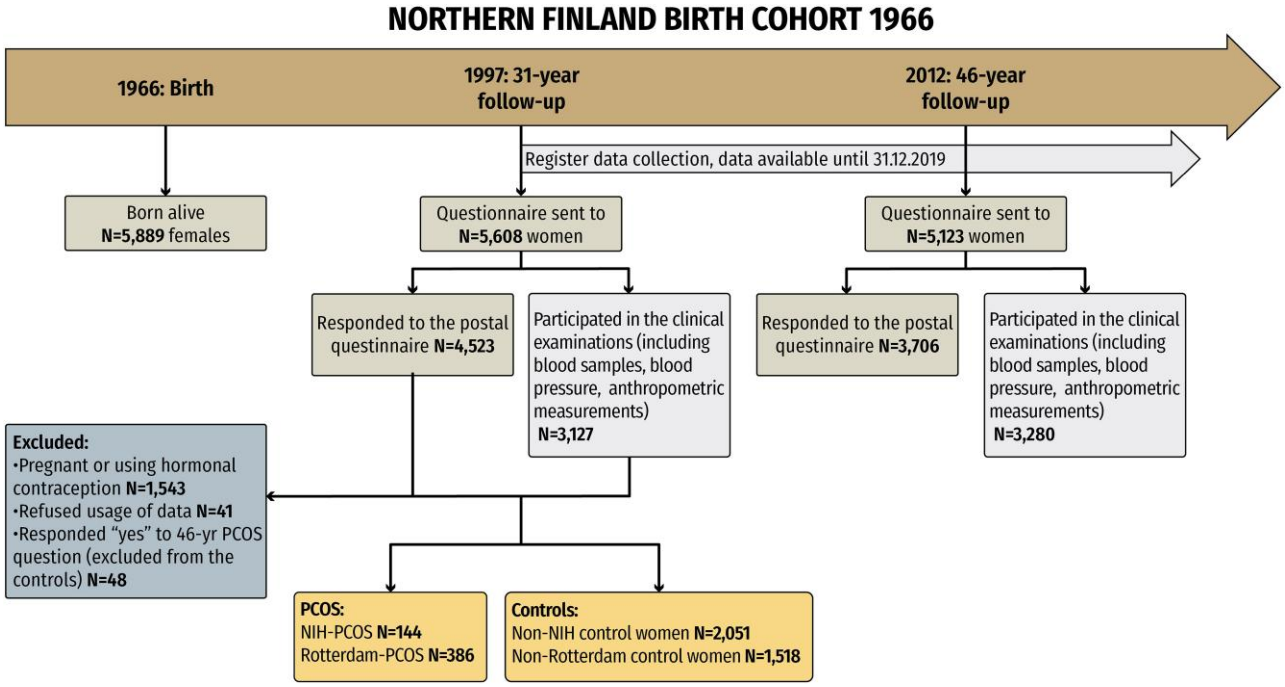

Figure 1. The flowchart of the Northern Finland Birth Cohort 1966.
PCOS, polycystic ovary syndrome, NIH, National Institute of Health.

2023). Indeed, AMH has been well documented as a surrogate for ovarian follicle count in ultrasonography and particularly the cut-off of 3.2 ng/mL for PCOM has been validated by Dietz de Loos *et al* using the same AMH assay in subjects of similar age as in this study.[19,20]

The presence of OA was screened by a postal questionnaire with the question "Is your menstrual cycle often [more than twice a year] longer than 35 days?" with the answers "Yes" or "My periods are missing completely" being considered as OA. The presence of clinical HA (hirsutism) was screened with the question "Do you have bothersome, excessive body hair growth?". We and others have previously shown that self-reported OA and HA can accurately identify women with PCOS.[6,21–23] Biochemical HA was defined as elevated testosterone (T) or free androgen index (FAI) levels based on our laboratory's reference ranges (T > 2.3 nmol/L or FAI >5.6). Women who, at age 31, were using hormonal contraceptives or were pregnant were excluded from the analysis.

Figure S1 describes the follow-up of women with NIH-PCOS and Rotterdam-PCOS.

Definition of control women

The women with Rotterdam-PCOS were compared to control women who did not have OA or clinical or biochemical HA and had AMH <3.2 ng/mL at age 31 ($n = 1518$). The women with NIH-PCOS were compared to control women who did not have OA or clinical or biochemical HA ($n = 2051$). The two different control groups were used because, in defining PCOS, the Rotterdam criteria account for PCOM, whereas NIH criteria does not, thus leading to different definitions of women without PCOS. Also, women who later reported being diagnosed with PCO/PCOS ($n = 48$, in the follow-up study at age 46) were excluded from the control groups.

Definition of outcome variables

CVD events

In Finland, public special health care (in tertiary referral hospitals) is affordable and accessible for everyone with serious acute illness, such as stroke or myocardial infarction (MI), and all hospitals report the diagnosis to the hospital discharge and hospital outpatient clinic registers. Every individual in Finland has a unique personal identification number enabling linkage to national registers. MI, stroke, transient ischemic attack (TIA) and heart failure were identified using the International Classification of Diseases, Revisions 8, 9 and 10 diagnostic codes (ICD-8, ICD-9 and ICD-10, respectively) extracted from the hospital discharge, hospital outpatient and basic healthcare registers. The registers covered the years 1972 to 2018, 1998 to 2018 and 2011 to 2019, respectively. Cardiovascular mortality data were retrieved from the mortality register of Statistics Finland, which covers information about the cause of death and time of death for every deceased individual in Finland. The primary endpoint of this study, a major adverse cardiovascular event (MACE), was defined as a composite endpoint incorporating MI, stroke, heart failure and cardiovascular mortality (primary cause of death I00-I99).

Blood pressure

In the clinical examinations, brachial systolic and diastolic BP was measured twice at age 31 and three times at age 46 with a 1-minute interval after 15 minutes of rest of the seated participants using a manual mercury sphygmomanometer at age 31 and an automated, oscillometric BP device (Omron Digital Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan) at age 46.

Type 2 diabetes mellitus (T2DM) and glucose metabolism

At age 46, a 2-hour oral glucose tolerance test was performed after overnight (12-hour) fasting in 2780 women. Plasma glucose levels were measured at the baseline and at 30, 60 and 120 minutes after the 75-g glucose load and classified according to World Health Organization standards into normal glucose tolerance (NGT), pre-diabetes (impaired fasting glucose or impaired glucose tolerance) and type 2 diabetes mellitus.[24] Also, information about previously diagnosed T2DM was gathered from the postal questionnaire at age 46. All self-reported T2DM diagnoses were verified and completed from the hospital discharge registers and the national drug registers from the Social Insurance Institution of Finland.

Fasting plasma glucose and insulin levels were measured at ages 31 and 46, and these values were used to calculate the homeostatic model assessment-insulin resistance (HOMA-IR) and the homeostatic model assessment of insulin of β-cell function (HOMA-B) indexes both at ages 31 and 46. The following equations were used: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5, and HOMA-B = 20 × insulin(μU/mL)/[Glucose (mmol/L−3.5].

Metabolic syndrome

Cholesterol levels were measured both at age 31 and 46. The presence of metabolic syndrome at age 46 was defined according to the International Diabetes Federation criteria. These criteria require central obesity (≥80 cm for Europid women) plus any two of the following four factors: triglyceride levels above 1.7 mmol/L or specific treatment for this lipid abnormality, high-density lipoprotein (HDL) below 1.3 mmol/L or specific treatment, increased blood pressure (systolic BP ≥130 or diastolic BP ≥85 mmHg) or treatment of previously diagnosed hypertension, or raised fasting plasma glucose (≥5.6 mmol/L) or previously diagnosed T2DM.

Medication

The use of antihypertensive medication or cholesterol-lowering medication was based on self-reported information from the postal questionnaire at age 46.

Laboratory methods

The serum levels of T, sex-hormone binding globulin (SHBG) and insulin, as well as plasma glucose levels, were analysed in NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189), as previously described in detail.[7] Briefly, the serum T levels were assayed using liquid chromatography-mass spectrometry equipment (Agilent Technologies, Wilmington, DE, USA) and SHBG by fluoroimmunoassay (Wallac, Inc. Ltd., Turku, Finland). FAI levels were calculated using the formula FAI = 100×T (nmol/L)/SHBG(nmol/L). Serum AMH levels were retrospectively assayed in 2020 by Elecsys® AMH Plus electrochemiluminescence immunoassay (Roche Diagnostics, Germany).[18] Serum total cholesterol, HDL, low-density lipoprotein (LDL) and triglycerides were determined using enzymatic assay methods (Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

Statistical methods

Data were analysed using IBM SPSS Statistics version 27 (IBM Corporation, Armonk, NY). The normality of the variables was checked visually. Normally distributed variables were presented as means with standard deviations, and skewed data as medians with 25th and 75th percentiles. A two-sided t-test or Mann-Whitney U-test was used to test differences between group characteristics. A *P*-value <.05 was considered statistically significant. The overall prevalence of MACE and individual endpoints was calculated based on the follow-up date until December 31, 2019.

Cox regression analysis was used to study the hazard ratio (HR) with 95% confidence intervals for the occurrence of CVD events. In Cox regression, the follow-up begun in 1997, as the identification of PCOS cases and their controls was made at the follow-up examination in 1997. None of the cohort members had MACE before 1997 ie, before baseline. In the Cox regression, the follow-up ended at the time of MACE or on December 31, 2018. In Cox regression, the end of follow-up was set to December 31, 2018, because most of the register data were available until that time, and thus majority of individuals would be censored on that date (only the basic health care register was available until December 31, 2019). Both crude and BMI-adjusted (age 31) HRs were calculated. The models were adjusted for BMI, which is known to be causally associated with both PCOS and CVD. Cox regression models were performed in multiple imputed datasets. In the imputed datasets, for example, BMI information was predicted by waist and hip circumference at age 31 and BMI at age 46. Fully conditional specification and predictive mean matching were used in the imputation.

Ethical approval

The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern Ostrobothnia Hospital District approved the research (decision number 94/2011). All participants took part on a voluntary basis and signed an informed consent form.

Results

Baseline characteristics at age 31

As shown in Table 1, both women with NIH-PCOS and women with Rotterdam-PCOS displayed significantly elevated systolic and diastolic BP as well as higher levels of LDL, triglycerides, insulin, HOMA-IR and HOMA-B, and a significantly lower level of HDL, when compared to control women. Women with Rotterdam-PCOS also had significantly higher levels of total cholesterol compared to control women, whereas there was no significant difference between NIH-PCOS and control women; however, this is likely due to the lower number of participants in this group. Alcohol consumption, smoking habits and education were comparable between women with NIH-PCOS and women with Rotterdam-PCOS compared to control women.

Cardiovascular disease (CVD) events

MACE

Both women with NIH-PCOS and women with Rotterdam-PCOS had a significantly higher risk for cardiovascular events than control women (Table 2). The pooled crude HR for MACE in the Rotterdam-PCOS group was 2.86 (1.58-5.16), and the BMI-adjusted pooled HR was 2.33 (1.26-4.30). The

Table 1. Baseline characteristics at age 31 in women with NIH-PCOS, women with Rotterdam-PCOS and control women.

At age 31	Control women (n = 1316-1552)	NIH-PCOS (n = 88-104)	*P*-value[a]	Control women (n = 934-1122)	Rotterdam-PCOS (n = 261-304)	*P*-value[b]
Systolic BP (mmHg)	118.78 ± 11.8	124.60 ± 13.6	<.001	118.70 ± 11.5	121.10 ± 13.6	.005
Diastolic BP (mmHg)	74.14 ± 10.1	79.28 ± 12.0	<.001	73.98 ± 10.1	77.10 ± 11.4	<.001
Total cholesterol (mmol/L)	4.81 ± 0.9	4.95 ± 0.9	.129	4.80 ± 0.9	4.95 ± 0.9	.009
LDL (mmol/L)	2.76 ± 0.8	2.95 ± 0.8	.023	2.77 ± 0.8	2.92 ± 0.8	.006
HDL (mmol/L)	1.67 ± 0.4	1.51 ± 0.3	<.001	1.65 ± 0.4	1.58 ± 0.3	.002
Triglycerides (mmol/L)	0.89 ± 0.4	1.14 ± 0.6	<.001	0.90 ± 0.5	1.03 ± 0.6	<.001
Glucose (mmol/L)	4.91 ± 0.5	4.50 ± 0.4	.081	4.91 ± 0.5	4.93 ± 0.4	.400
Insulin (mIU/L)	7.60 ± 3.1	10.15 ± 5.4	<.001	7.68 ± 3.4	8.91 ± 4.9	<.001
HOMA-IR	0.98 ± 0.4	1.31 ± 0.7	<.001	0.99 ± 0.5	1.15 ± 0.6	<.001
HOMA-B	97.48 ± 23.4	111.96 ± 36.0	<.001	98.02 ± 24.3	105.47 ± 31.4	<.001
Alcohol consumption (g/day)	2.20 [0.60; 5.90]	1.75 [0.43; 6.65]	.641	2.30 [0.60; 6.20]	1.70 [0.50; 5.80]	.095
Smoking			.110			.217
Never smoker	40.9% (n = 829)	39.5% (n = 58)		41.9% (n = 631)	39.4% (n = 152)	
Former smoker >6 mo	22.1% (n = 448)	26.5% (n = 39)		22.2% (n = 334)	21.8% (n = 84)	
Former smoker <6 mo	11.9% (n = 241)	6.1% (n = 9)		11.6% (n = 174)	9.6% (n = 37)	
Current smoker	25.1% (n = 508)	27.9% (n = 41)		24.3% (n = 366)	29.3% (n = 113)	
Education			.806			.093
Basic	8.8% (n = 180)	9.6% (n = 14)		8.7% (n = 132)	8.7% (n = 34)	
Secondary	70.6% (n = 1447)	71.9% (n = 105)		69.4% (n = 1053)	74.3% (n = 289)	
Tertiary	20.6% (n = 423)	18.5% (n = 27)		21.9% (n = 333)	17.0% (n = 66)	

Results are reported as mean with SD or median with [25%; 75%] quartiles. The number of cases in the groups varied due to missing data for some variables. The difference between groups was tested by the Student's t-test or Mann-Whitney U-test, when appropriate.
Fasting plasma glucose and fasting serum insulin values were used to calculate the homeostatic model assessment-insulin resistance (HOMA-IR) index with the following formula: fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5. The homeostatic model assessment of insulin of β-cell function (HOMA-B) was calculated using the equation 20 × insulin(μU/mL)/[Glucose (mmol/L) – 3.5] to quantify pancreatic β-cell insulin secretion.
Abbreviations: BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; mo, months.
[a]Between NIH-PCOS and control women.
[b]Between Rotterdam-PCOS and control women.

Table 2. The prevalence as well as the crude and body mass index adjusted Cox regression analysis for cardiovascular disease events in women with NIH-PCOS, Rotterdam-PCOS and respective control women.

	Prevalence and number of events	*P*-value	Crude HR (95% CI)	BMI adjusted HR (95% CI)
Outcome: MACE				
Non-NIH control women	2.2% (43/1973)	<.001	1.00	1.00
NIH-PCOS	7.8% (11/141)		3.49 (1.75-6.96), $P < .001$	2.47 (1.18-5.17), $P = .016$
Non-Rotterdam control women	1.8% (27/1469)	<.001	1.0	1.0
Rotterdam-PCOS	5.2% (20/382)		2.86 (1.58-5.16), $P < .001$	2.33 (1.26-4.30), $P = .007$
Outcome: Myocardial infarction				
Non-NIH control women	0.8% (16/2001)	.010	1.00	1.00
NIH-PCOS	3.5% (5/141)		2.73 (.79-9.35), $P = .111$	2.20 (.60-8.08), $P = .236$
Non-Rotterdam control women	0.7% (10/1469)	.019	1.00	1.00
Rotterdam-PCOS	2.1% (8/382)		2.34 (.85-6.43), $P = .100$	1.85 (.64-5.33), $P = .255$
Outcome: Stroke				
Non-NIH control women	1.1% (23/2001)	.097	1.00	1.00
NIH-PCOS	NA % (<5/141)		2.60 (.89-7.53), $P = .079$	1.81 (.58-5.66), $P = .309$
Non-Rotterdam control women	1.0% (15/1469)	.024	1.00	1.00
Rotterdam-PCOS	2.6% (10/382)		2.76 (1.22-6.20), $P = .014$	2.59 (1.13-5.96), $P = .025$

A major adverse cardiovascular event (MACE) defined as a composite endpoint incorporating myocardial infarction, stroke, heart failure and cardiovascular mortality.
If there were fewer than five cases per group, the data is reported as "<5" to ensure data protection and study participants' anonymity. Women with NIH-PCOS are compared to non-NIH control women, and women with Rotterdam-PCOS are compared to non-Rotterdam control women.
Abbreviations: NIH, National Institute of Health; PCOS, Polycystic ovary syndrome; HR, Hazard ratio; 95% CI, 95% confidence intervals; BMI, body mass index.

pooled crude HR for MACE in the NIH-PCOS group was 3.49 (1.75-6.96), and the pooled BMI-adjusted HR was 2.47 (1.18-5.17). The cumulative hazard curves began to diverge as early as age 35 (Figure 2). Also, BMI at age 31 was a significant risk factor for MACE (hazard ratios for BMI were 1.09 [1.04-1.13] and 1.08 [1.04-1.13] in the NIH-PCOS multivariate analysis and in the Rotterdam-PCOS multivariate analysis, respectively). At age 53, the prevalence of MACE was significantly higher in NIH-PCOS and Rotterdam-PCOS groups when compared to control women (Table 2).

Individual CVD endpoints

As shown in Table 2, when compared to control women, MI was significantly more prevalent in both women with NIH-PCOS and women with Rotterdam-PCOS. However, the hazard ratios for MI in the Cox regression analysis were not significant in either PCOS group, possibly due to the small number of events. The prevalence as well as the pooled crude and BMI-adjusted HRs for stroke were significantly increased in women with Rotterdam-PCOS but not in women with NIH-PCOS, when compared to control women (Table 2). However, the NIH-PCOS group might have been underpowered to detect the difference. The occurrence of heart failure as well as cardiovascular disease mortality were rare in both NIH-PCOS and Rotterdam-PCOS groups and did not significantly differ from the control women (data not shown to ensure data protection and study participant's anonymity).

Blood pressure and use of antihypertensive medication at age 46

Women with Rotterdam-PCOS had significantly elevated systolic and diastolic BP at age 46, whereas women with NIH-PCOS had only significantly elevated diastolic BP (Table 3). However, the actual systolic BP values were very similar in both PCOS groups, which suggests that the NIH group was underpowered to detect the difference in systolic BP. Moreover, 30.7% ($n = 31/101$) of women with NIH-PCOS and 23.4% ($n = 69/295$) of women with Rotterdam-PCOS used antihypertensive medication compared to about 15% of control women (Figure 3).

Cholesterol levels and use of cholesterol-lowering medication at age 46

Women with NIH-PCOS used cholesterol-lowering medication significantly more often than control women (Figure 3). Both women with NIH-PCOS and women with Rotterdam-PCOS had significantly higher levels of LDL and significantly lower levels of HDL compared to control women (Table 3).

Glucose metabolism and metabolic syndrome at age 46

T2DM, prediabetes and metabolic syndrome were significantly more prevalent among both the Rotterdam-PCOS and NIH-PCOS groups compared to control women (Figure 3).

Sub-analysis of non-NIH Rotterdam-PCOS groups

Women with PCOS phenotype C (HA + PCOM) had significantly higher BMI, waist circumference, systolic and diastolic BP, and serum levels of T and FAI at age 46 compared to control women (Table 4). Women with PCOS phenotype D (OA + PCOM) had significantly lower serum SHBG and HDL levels at age 46 compared to control women. Due to the smaller size of the PCOS phenotype C and D groups, the groups were combined to present a non-NIH Rotterdam-PCOS group, simultaneously bringing more power to the statistical analysis. The prevalence of MACE was 3.8% ($n = 9/240$) in the combined C + D (ie, non-NIH) phenotype PCOS group compared to 2.1% in control women ($P = .083$). The risk for MACE in the C or D PCOS phenotype was not significant, even after pooling the groups for the analysis (crude pooled HR = 2.13 [0.999-4.55], BMI-adjusted pooled HR = 1.89 [0.87-4.07]).

Discussion

The main finding of this large, prospective, population-based cohort study was that women with PCOS, regardless of whether they were diagnosed according to the NIH or

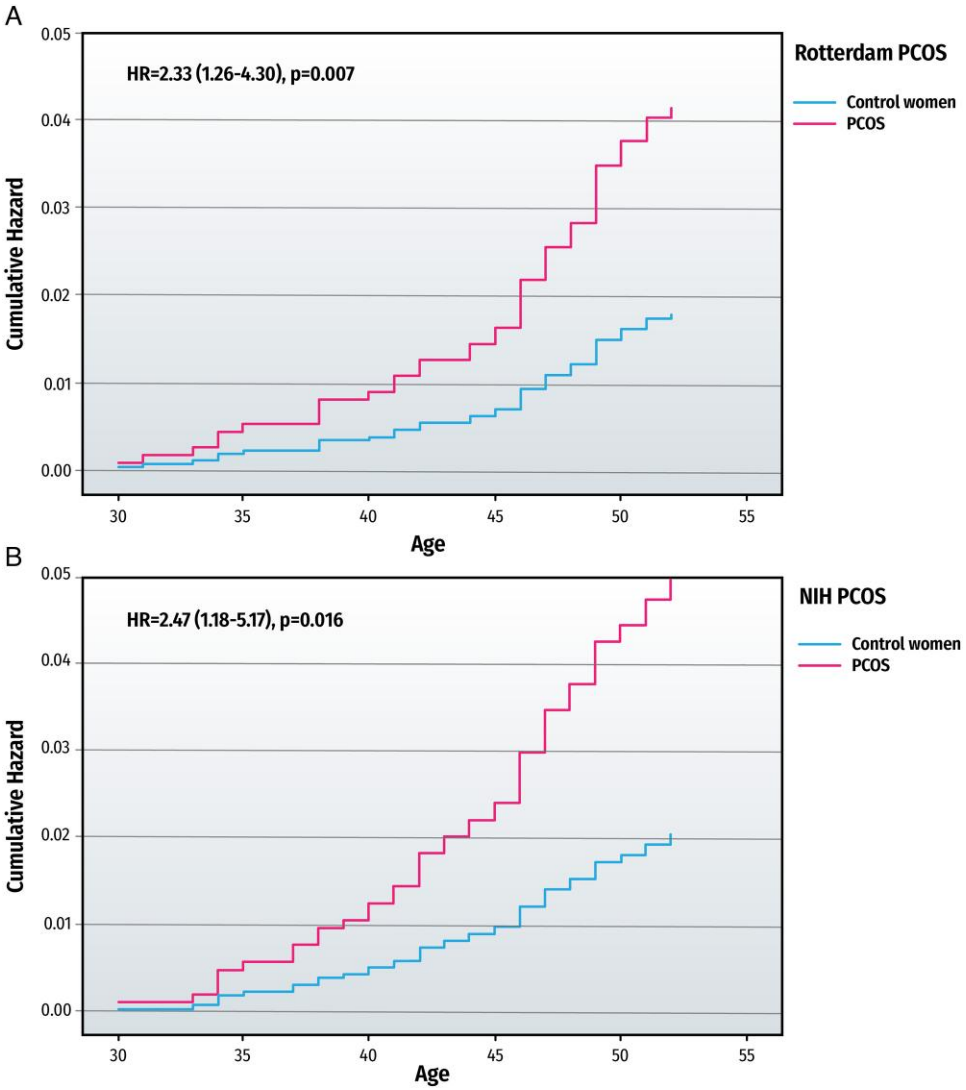

Figure 2. The Cox proportional hazard functions for major adverse cardiovascular events in women with PCOS according to the Rotterdam criteria (A) and NIH criteria (B). PCOS, polycystic ovary syndrome.
The analysis was adjusted by body mass index at age 31.

Table 3. Hormonal and metabolic parameters at age 46 in women with NIH-PCOS, women with Rotterdam PCOS and control women.

At age 46	Control women (n = 1290-1514)	NIH-PCOS (n = 85-101)	P-value[a]	Control women (n = 910-1085)	Rotterdam-PCOS (n = 256-299)	P-value[b]
Testosterone (nmol/L)	0.85 ± 0.3	0.97 ± 0.3	.001	0.85 ± 0.3	0.92 ± 0.4	.006
SHBG (nmol/L)	54.9 [38.5; 75.7]	48.1 [33.8; 60.9]	.002	55.2 [38.6; 75.4]	48.8 [34.9; 64.8]	<.001
FAI	1.47 [1.04; 2.12]	1.94 [1.51; 2.70]	<.001	1.46 [1.05; 2.08]	1.81 [1.34; 2.46]	<.001
BMI (kg/m^2)	26.3 ± 5.2	28.9 ± 5.7	<.001	26.3 ± 5.2	27.9 ± 5.8	<.001
Waist (cm)	86.5 ± 12.9	92.9 ± 14.2	<.001	86.6 ± 12.9	91.0 ± 14.5	<.001
Systolic BP (mmHg)	119.74 ± 15.5	122.52 ± 16.3	.073	119.44 ± 15.0	122.23 ± 16.0	.005
Diastolic BP (mmHg)	81.83 ± 10.5	84.70 ± 10.3	.005	81.72 ± 10.4	84.03 ± 11.4	.001
Total cholesterol (mmol/L)	5.17 ± 0.9	5.22 ± 0.8	.671	5.22 ± 0.9	5.23 ± 0.9	.621
LDL (mmol/L)	3.23 ± 0.9	3.42 ± 0.8	.047	3.24 ± 0.9	3.38 ± 0.9	.035
HDL (mmol/L)	1.67 ± 0.4	1.53 ± 0.3	.001	1.68 ± 0.4	1.57 ± 0.4	<.001
Triglycerides (mmol/L)	1.08 ± 0.6	1.16 ± 0.5	.224	1.09 ± 0.6	1.15 ± 0.6	.144

Results are reported as mean with SD or median with [25%; 75%] quartiles. The number of cases in the groups varied due to missing data for some variables. The difference between groups was tested by the Student's t-test or Mann-Whitney U-test, when appropriate.
Abbreviations: T, testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein.
[a]Between NIH-PCOS and control women.
[b]Between Rotterdam-PCOS and control women.

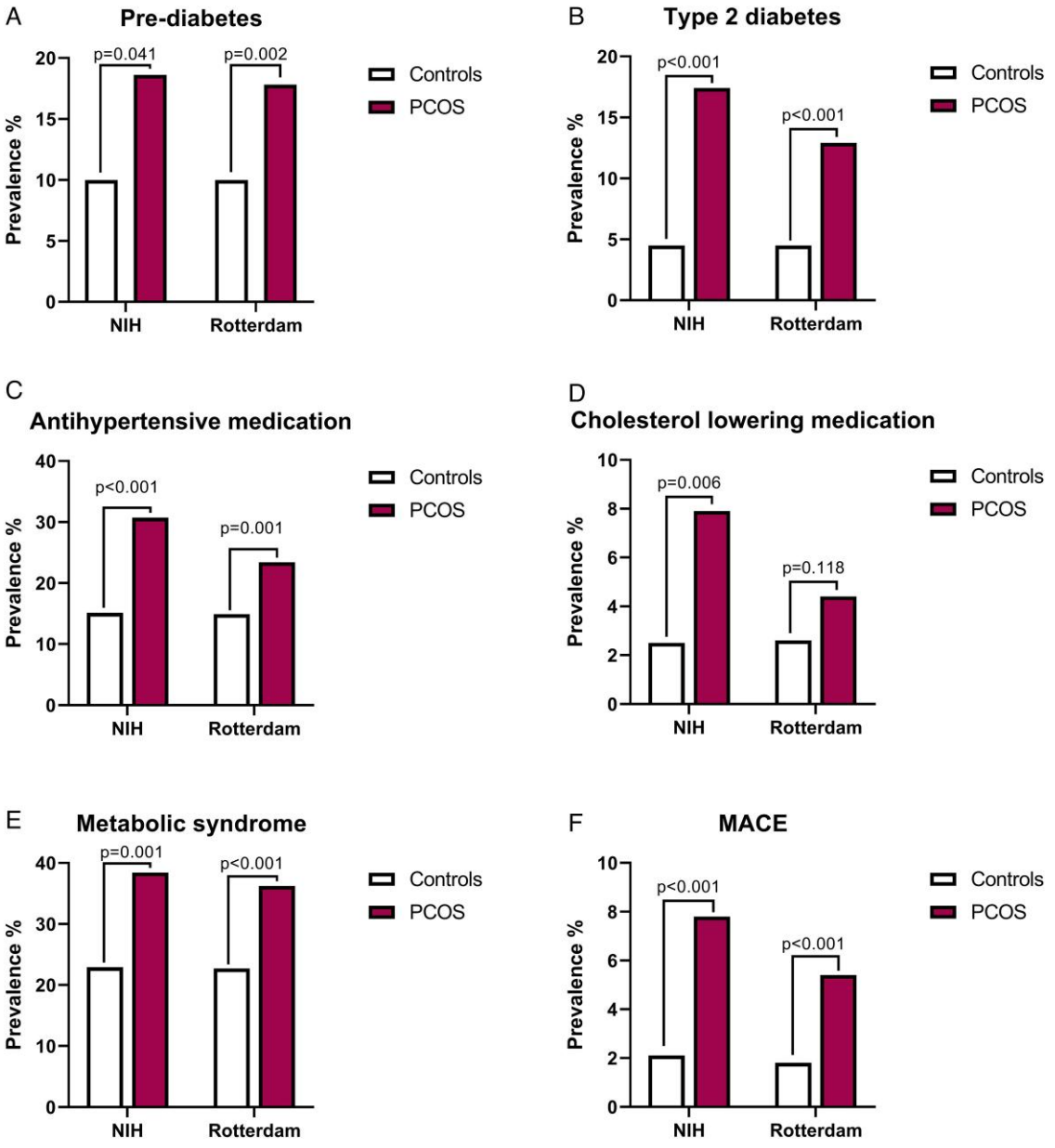

Figure 3. The prevalence of pre-diabetes (A), type 2 diabetes (B), the use of antihypertensive medication (C), the use of cholesterol lowering medication (D), metabolic syndrome (E), and MACE (F) in control women and in women with PCOS diagnosed according to the Rotterdam criteria or NIH criteria. PCOS, polycystic ovary syndrome, MACE, Major adverse cardiovascular event.
The difference in prevalence was analysed using the Chi-square test.

Rotterdam criteria, had a significantly increased risk of major CVD events. We also found that the risk for MACE already started to deviate from the control women during early adulthood and that the increased risk of CVD was independent of BMI. These findings indicate that PCOS should be considered a major risk factor for CVD.

Very few prospective cohort studies have investigated the risk of CVD events in women with PCOS.[13,25,26] A recent systematic review[27] included only three prospective cohort studies that had investigated the risk of CVD events, such as non-fatal or fatal coronary artery or cerebrovascular diseases. Of these three studies, two included hospital-based study populations,[13,25] whereas only one was a population-based, prospective cohort study.[26] Thus, the present study significantly adds to the literature regarding knowledge of PCOS-related CVD risk in the general population. Moreover, our study was the first to investigate the risk of CVD events using both the NIH-PCOS and Rotterdam-PCOS criteria.

Previous prospective cohort studies have not found a statistically significant increase in the risk of CVD events in women with PCOS.[13,25,26] However, this might have been due to the studies being too underpowered to detect significant differences, as noted by Schmidt *et al*. Indeed, Schmidt *et al* evaluated the CVD event risk of 32 women with PCOS,[13] whereas Merz *et al* evaluated 25 women with PCOS.[25] In fact, Schmidt *et al* found that women with PCOS had an over 10-percent higher prevalence of CVD events (28.1% versus 16.8%) than control women, but this did not reach statistical significance.[13] Similarly, Merz *et al* found that women with PCOS tended to more often have multivessel coronary artery disease (42% versus 27%, $P = .07$).[25] In the present study, we were able to evaluate 144 women with NIH-PCOS and 386 women with

Table 4. Hormonal and metabolic parameters at age 46 in control women and women with PCOS phenotypes C and D.

At age 46	Control women (n = 910-1085)	PCOS phenotype C (n = 89-90)	P-value[a]	PCOS phenotype D (n = 81-97)	P-value[b]
Testosterone (nmol/L)	0.85 ± 0.3	0.97 ± 0.4	.009	0.80 ± 0.3	.234
SHBG (nmol/L)	55.1 [38.6; 75.1]	50.1 [35.8; 72.2]	.218	47.9 [34.5; 63.6]	.007
FAI	1.47 [1.05; 2.07]	1.81 [1.33; 2.48]	.001	1.59 [1.18; 2.28]	.206
BMI (kg/m^2)	26.3 ± 5.3	27.5 ± 5.9	.040	27.1 ± 5.1	.180
Waist (cm)	86.6 ± 13.0	90.4 ± 15.2	.009	89.2 ± 13.0	.087
Systolic BP (mmHg)	120.3 ± 15.4	124.1 ± 19.0	.027	122.1 ± 15.3	.303
Diastolic BP (mmHg)	82.5 ± 10.7	85.1 ± 13.2	.034	84.04 ± 11.7	.230
Total cholesterol (mmol/L)	5.20 ± 0.9	5.31 ± 0.9	.280	5.17 ± 0.8	.732
LDL (mmol/L)	3.24 ± 0.9	3.39 ± 0.9	.138	3.31 ± 0.9	.504
HDL (mmol/L)	1.68 ± 0.4	1.62 ± 0.4	.181	1.57 ± 0.4	.030
Triglycerides (mmol/L)	1.09 ± 0.6	1.19 ± 0.8	.150	1.11 ± 0.5	.777

Results are reported as mean with SD or median with [25%; 75%] quartiles. The number of cases in the groups varied due to missing data for some variables. The difference between groups was tested by the Student's t-test or Mann-Whitney U-test, when appropriate.
Abbreviations: SHBG, sex hormone binding globulin; FAI, free androgen index; BMI, body mass index; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein.
[a]Between PCOS phenotype C (hyperandrogenism and AMH 3.2 ng/mL cut-off as a surrogate for PCOM) and control women.
[b]Between PCOS phenotype D (oligo-amenorrhea and AMH 3.2 ng/mL cut-off as a surrogate for PCOM) and control women.

Rotterdam-PCOS and thus our study population provides more power in statistical analyses.

Our results are in line with previous large, register-based studies from Denmark[11] and the United Kingdom[9] that showed an increased risk of CVD events in women with PCOS. Berni *et al* extracted data from the Clinical Practice Research Datalink Aurum database and found that the adjusted HR for MACE was 1.26 (95% CI: 1.13-1.41).[9] They also found that the risk of MI, angina and revascularization were increased, even though they studied young patients with a median age of 29 years.[9] Glintborg *et al* used nationwide register data of women with PCOS aged between 12 and 60 years. They found that the HR for the development of CVD in PCOS was 1.7 (95% CI: 1.7-1.8).[11] However, the definition of CVD in that study was rather wide, including CVD risk factors/mediating factors and venous thrombosis, and not just hard CVD endpoints.

In the present study, the well-known traditional CVD risk factors—pre-diabetes, T2DM, hypertension and metabolic syndrome—were significantly more prevalent in women with both NIH-PCOS and Rotterdam-PCOS. These findings are in line with our previous studies from this cohort using a self-reported PCOS population[5,7] as well as with the results of other authors.[27,28] The present study especially adds to the literature on the comparison of the prevalence of PCOS using both NIH and Rotterdam criteria. In the current study population, it was especially concerning that the use of cholesterol-lowering medication was markedly less common than the prevalence of MACE, as lowering cholesterol levels is one of the cornerstones of the secondary prevention of CVD. The international evidence-based guideline for the management of PCOS recommends the assessment of the lipid profile in overweight and obese women with PCOS at diagnosis and thereafter depending on the presence of dyslipidaemia and global CVD risk.[2] The present study strongly encourages regular follow-up of lipid levels in this high-risk population and active treatment when recommended levels are not met. The finding that PCOS phenotypes C and D present with alterations in the metabolic profile that do not seem to translate into increased risk of MACE warrants further evaluation. Especially, women with phenotype D showed only a few rather mild metabolic changes, and a longer follow-up time may be needed to determine whether these changes increase the risk of CVD events over time.

The major strengths of this study were its prospective, general population-based design and high participation rate. These strengths minimise the possibility of selection bias and provide an estimate that applies to the general population. Moreover, Finnish healthcare registers, from which the CVD event codes were retrieved, are of top quality in terms of coverage and accuracy.[29] We were also able to adjust for the effect of BMI, which is known to be causally associated with both PCOS and CVD. The limitations of this work include the relatively young age of the study subjects, and thus this population will continue to be followed in the future to assess how the risk of CVD events develops with aging. Moreover, for practical reasons, it was impossible to perform a transvaginal ultrasound assessment of polycystic ovarian morphology for the whole study population. This could be considered as a limitation, although AMH has been well documented as a surrogate for ovarian follicle count in ultrasonography and particularly the cut-off of 3.2 ng/mL for PCOM by Dietz de Loos *et al*.[19,20] Importantly, in this dataset, AMH was able to provide a more accurate estimate of antral follicle count than possible via ultrasound when the data were collected back in 1997. Of note, the AMH cut-off may vary depending on the AMH assay as well as the age and ethnicity of the study population.

Conclusion

Women with PCOS have an increased risk of MACE, regardless of whether PCOS was diagnosed according to the NIH or Rotterdam criteria. The fact that this risk already started to increase at the age of 35 indicates significant lifelong exposure to different CVD risk factors. Here, PCOS increased the risk of CVD independently of BMI; however, BMI *per se* was also a significant risk factor for CVD. The long-term follow-up beyond menopause will show how the risk of CVD events develops with aging. In the future, randomised controlled trials and real-world data are needed to investigate the most cost-effective methods for the primary and secondary prevention of CVD in women with PCOS.

Acknowledgments

We thank all cohort members and researchers who participated in the study. We also wish to acknowledge the work of the NFBC project centre.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

Funding

This study was funded by the Academy of Finland (315921, 321763, Profi6 336449), the Sigrid Juselius Foundation, Diabetestutkimussäätiö, Sakari Alhopuro Foundation, the Medical Research Center Oulu, the Novo Nordisk Foundation and Roche Diagnostics International Ltd. The NFBC1966 31-year follow-up received financial support from the University of Oulu Grant no. 65354, Oulu University Hospital Grant no. 2/97 and 8/97, Ministry of Health and Social Affairs Grant no. 23/251/97, 160/97 and 190/97, the National Institute for Health and Welfare, Helsinki Grant no. 54121 and the Regional Institute of Occupational Health, Oulu, Finland Grant no. 50621 and 54231. The NFBC1966 46-year follow-up received financial support from University of Oulu Grant no. 24000692, Oulu University Hospital Grant no. 24301140 and European Regional Development Fund (ERDF) Grant no. 539/2010 A31592. The data generation, curation and labour were also supported by the following EU H2020 grants: DynaHEALTH (633595), LifeCycle (733206), LongITools (873749), EarlyCause, EDCMET (825762) and the Medical Research Council, UK: grant number MRC/BBSRC MR/S03658X/1 (JPI HDHL H2020).

Conflicts of interest: Terhi Piltonen is on the editorial board of EJE. They were not involved in the review or editorial process for this paper, on which they are listed as authors. Other authors have nothing to declare.

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