**Association of self-reported polycystic ovary syndrome, obesity and weight gain from
adolescence to adulthood with hypertensive disorders of pregnancy
-a community-based approach**

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39 **Abbreviations:** Body mass index (BMI), confidence interval (CI), Finnish Medical Birth
40 Register (FMBR), free androgen index (FAI), hirsutism (H), hypertensive disorder of
41 pregnancy (HDP), interquartile range (IQR), Northern Finland birth cohort 1966 (NFBC1966),
42 odds ratio (OR), oligo-amenorrhea (OA), polycystic ovaries (PCO), polycystic ovary syndrome
43 (PCOS), pregnancy induced hypertension (PIH), sex hormone binding globulin (SHBG),
44 testosterone (T)

ABSTRACT

The purpose of this prospective, population-based cohort study was to evaluate the roles of polycystic ovary syndrome (PCOS), obesity, weight gain and hyperandrogenemia in the development of hypertensive disorders of pregnancy (HDP) through fertile age both in PCOS and in non-PCOS women. The study population, the Northern Finland Birth Cohort 1966, allowed a long-term follow-up of women from age 14 until 46 who developed HDP (n=408) or did not (n=3373). HDP diagnosis was confirmed by combining hospital discharge records, data from Finnish Medical Birth Registers and the questionnaire data at age 46. Women with self-reported PCOS (srPCOS, n=279), defined by both oligo-amenorrhea and hirsutism at age 31 and/or with PCOS diagnosis by age 46, were compared with women without reported PCOS (n=1577). Women with srPCOS had an increased HDP risk (OR=1.56 [95%CI:1.03-2.37]), but the association disappeared after adjustment for BMI. In women with srPCOS and HDP, BMI increased from age 14 to age 46 significantly more than in srPCOS women without HDP (median [interquartile range]: 9.82kg/m² [6.23;14.6] and 7.21kg/m² [4.16;10.5], respectively, p<0.001). Also, in non-PCOS women, the increase was higher in women with (7.54kg/m² [5.32;11.62], p<0.001) than without HDP (6.33kg/m² [3.90;9.33], p<0.001). Increase in waist circumference between ages 31 and 46 was associated with HDP, but not with PCOS. Hyperandrogenemia at 31 or 46 did not associate with HDP (1.44;0.98-2.11). In conclusion, obesity, especially abdominal obesity, and weight gain from adolescence to age 46, but not srPCOS or hyperandrogenemia, were associated with an increased risk of HDP.

Keywords: Cohort study, hyperandrogenemia, hypertensive disorders of pregnancy, PCOS, preeclampsia, obesity, weight gain

68 INTRODUCTION

69 Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 5 to 18% of fertile-aged
70 women.¹⁻³ It is defined by the presence of two or more of the following criteria: (i) polycystic
71 ovaries (PCO); (ii) oligo-amenorrhea (OA) or amenorrhea; and/or (iii) clinical or biochemical
72 evidence of hyperandrogenism.^{4,5} The syndrome is associated with overweight and obesity in
73 20% to 80% of the women depending on the population studied and diagnostic criteria used.^{6,7}

74 Hypertensive disorders of pregnancy (HDP) complicate 5-10% of all
75 pregnancies.⁹ Hypertensive disorders of pregnancy include chronic hypertension (blood
76 pressure of at least >140/90 mmHg before pregnancy or before 20 weeks of gestation),
77 preeclampsia (new onset of hypertension and proteinuria after 20 weeks of gestation),
78 superimposed preeclampsia (chronic hypertension in association with preeclampsia) and
79 gestational hypertension (defined according to the same criteria as preeclampsia but without
80 proteinuria).¹⁰⁻¹² All these disorders are associated with increased maternal and fetal morbidity
81 and mortality during pregnancy and can also affect the future health of both mother and child.¹³
82 Women with a history of HDP are at higher risk of chronic hypertension, dyslipidemia,
83 cardiovascular diseases, type 2 diabetes mellitus and kidney disease in later life.^{9,13}

84 Recent meta-analyses have suggested that PCOS is associated with an increased
85 risk of pregnancy induced hypertension (PIH) and pre-eclampsia,^{3,15-17} although conflicting
86 results have also been obtained.¹⁸⁻²⁰ Of note, in some studies, the association between PCOS
87 and HDP has been confounded by multiple factors such as a higher multiple pregnancy rate,
88 parity, age and body mass index (BMI).^{16,21} It has also been suggested that the increased risk
89 of HDP in PCOS may be linked mainly to obesity or hyperandrogenemia, but not specifically
90 to the syndrome itself.

This study had two main aims: firstly, to investigate the prevalence of HDP in women with PCOS and secondly, to evaluate the independent roles of PCOS, obesity, weight gain and hyperandrogenemia in the development of HDP through the fertile age.

METHODS

Data collection and study population

Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to NFBC project center at: (NFBCprojectcenter@oulu.fi) and researchers can visit the cohort website (www.oulu.fi/nfbc) for more information.

The study population consisted of the Northern Finland Birth Cohort 1966 (NFBC1966), a unique population-based, longitudinal cohort study of subjects in the two northernmost provinces of Finland. Pregnant women expected to deliver in 1966 were recruited if they were still pregnant at the 24th gestational week and they gave birth to 12058 alive children, of these 5889 were females. These subjects were followed up at multiple time-points, with the key data collections at ages 14, 31 and 46. At age 14, in 1980, the adolescent females (n=5455, 94.6%) answered a postal questionnaire, with the help of their parents, including questions about weight and height. 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) were invited to a clinical examination. Of these, 3127 (77%) women participated in a clinical examination including anthropometric measurements and blood samples for hormonal and metabolic parameters. Again, at age 46, a new large questionnaire including all main health issues and an invitation to clinical examination was sent to 5123 women. Of these, 3706 (72.3%) answered the questionnaire and 3280 women

(64.0%) participated in the clinical examinations, including blood sampling (Figure 1). When compiling the final present study population, women who had not delivered a child by age 46 (n=638) were excluded from the analyses.

In all clinical examinations, participants' weight (kg) was measured with a regularly calibrated, digital scale. Height (cm) was measured twice by using standard and calibrated stadiometer and the average of the measurements was calculated. BMI was calculated (kg/m^2) and women who were overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$) or obese ($\text{BMI} > 30 \text{ kg/m}^2$) were identified. BMI values at ages 31 and 46 from clinical examination and postal questionnaire were combined to create a variable where clinically measured BMI was primarily used and self-reported BMI if measured BMI was not available. The clinically measured and self-reported BMIs did not differ.^{22,23} Weight changes (median $\pm$ SD) between ages 14-31, 31-46 and 14-46 as well as increase in waist circumference between ages 31 and 46 were calculated in each study group. In addition, BMI values at adolescence were converted into age- and sex-specific BMI z-score by applying national reference data.²⁴ BMI z-scores at ages 31 and 46 were based on data of the whole female NFBC1966 population.

Blood pressure was measured with a calibrated device and at least two separate measurements were performed at least four to six hours apart.²⁵

The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern Ostrobothnia Hospital District (EETTMK decision number 94/2011) has approved the research on September 17th, 2012. All participants took part on a voluntary basis and signed an informed consent to use all the data.

Definition of HDP diagnosis

The diagnosis of HDP had to be assessed in at least two of the three following sources to be considered as reliable: the Finnish Medical Birth Register (FMBR), the hospital discharge register (HDR) or the questionnaire at age 46 (Figure S1 <http://hyper.ahajournals.org>).

Definition of PCOS diagnosis

At age 31, the questionnaire included two questions on oligo-amenorrhea (OA) and hirsutism (H): 1) is your menstruation cycle longer than 35 days more than twice a year (OA)? and 2) do you have excessive body hair (H)? Of the women who responded to the questionnaire (excluding women using hormonal contraception, n=1459 and those not permitting the use of their data for data analysis, n=41), 10.4% (n=321) reported isolated H, 10.2% (n=330) isolated OA and 3.4% (n=125) both OA and H. Women with only one PCOS symptom were excluded from the analyses. At age 46, the question on self-reported PCOS was inquired as follows: Have you been diagnosed with polycystic ovaries (PCO) and/or PCOS? Women with either 1) symptoms at age 31, 2) self-reported PCO/PCOS diagnosis by age 46, or 3) symptoms at age 31 and self-reported PCO/PCOS diagnosis by age 46 were classified as cases (self-reported PCOS, srPCOS, n=279). It has been previously shown that women with srPCOS present the typical hormonal, endocrine, metabolic and psychologic profile of PCOS.²⁶⁻²⁹ Women without any PCOS symptoms at age 31 and without self-reported diagnosis of PCOS by age 46 were classified as “non-PCOS women” (n=1577, Figure 1).

Final study population

The study population was further divided into four groups: women with srPCOS with HDP (n=36), women with srPCOS without HDP (n=154), non-PCOS women with HPD (n=161) and non-PCOS women without HDP (n=1045) (Figure 1).

Laboratory methods

Biochemical assays and laboratory methods used at age 31 have been detailed previously²⁸ and are described in more detail in <http://hyper.ahajournals.org>.

Statistical Analyses

The differences in distributions of clinical characteristics were tested by using nonparametric Mann-Whitney U test, when appropriate, otherwise a *t*-test was used. The p-values were further adjusted for BMI at ages 31 and 46 using univariate general linear modelling (ANCOVA). Categorical data were analyzed using cross-tabulation and Pearson's Chi-squared (χ^2) test. Continuous data are presented as medians with lower (25th) and upper quartiles (75th) (interquartile range, IQR).

The whole study population was also stratified into quartiles according to serum total T level and FAI at age 31 and 46. Chi-squared (χ^2) test's Linear-by-Linear association was used to identify the trend of HDP prevalence across these quartiles. The p-values were further adjusted for BMI at age 31 and 46 using a binary logistic regression model.

Binary logistic regression models were employed to estimate the factors associated with HDP. The models were adjusted for the consumption of alcohol, smoking and education status at age 46. The results are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs). A p-value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Association of srPCOS with HDP (Figure 2)

The reproductive lifetime prevalence (thereafter prevalence) of HDP was 14.1% (n=197/1396) in the whole population. Women with srPCOS presented more often with HDP compared with the non-PCOS women, 18.9% vs 13.3%, respectively (p=0.044) with 1.5 times higher risk of HDP (OR=1.56 [95% CI: 1.03-2.37]). The difference remained after adjustment for waist circumference both at ages of 31 and 46 but was abolished after adjustment for BMI (table 1).

The prevalence of HPD was similar in normal weight women (both in ages of 31 and 46 years) with or without srPCOS. However, in obese women, women with PCOS had significantly higher risk when compared with non-PCOS women (Figure 2).

Comparison of non-PCOS women with and without HDP (Tables 1A and 1B, Table S1, Figures 3, 4A, 4B and S2)

In the non-PCOS women, BMI was significantly higher in ages of 14, 31 and 46 and waist circumference was significantly greater at ages 31 and 46 in women with HDP when compared to those without HDP. The risk of HDP increased along with BMI class (at ages 31 and 46) (Table 1A, Table S1, Figure 3) and weight gain was significantly greater from adolescence to adulthood (age 46) in the HDP group (Table 1A, Table S1, Figures 4A and S2). The increase of waist circumference showed a divergent pattern but did not significantly differ between the two groups from 31 to 46 years (Figure 4B).

Comparison of women with srPCOS with and without HDP (Tables 1A and 1B, Table S1, Figures 4A, 4B and S2)

When women with srPCOS with and without HDP were compared, BMI was significantly higher at all time points and waist circumference was significantly greater at age 46 (p=0.003) in srPCOS cases with HDP than without HDP. Women with HDP also had greater weight gain from adolescence to age 46 (Table 1A, Table S1, Figures 4A and S2). As in non-PCOS women,

waist circumference showed a divergent pattern but did not significantly differ between the two groups (age 31-46) (Figure 4B). At ages 31 and 46, FAI was significantly higher in women with srPCOS and HDP ($p=0.015$ and $p=0.020$, respectively), but statistical significance was lost after adjustment for BMI (Table 1B).

Comparison of women with HDP with and without srPCOS (Tables 1A and 1B, Table S1, Figures 4A, 4B and S2)

In women with HDP, BMI was significantly higher in the srPCOS group than in those without srPCOS at all timepoints (Table 1A, Table S1, Figures 4A and S2). Waist circumference was significantly greater at ages 31 ($p=0.021$) and 46 ($p=0.011$) in women with PCOS than in those without. The increase of waist circumference between ages 31 and 46 did not differ between the two groups (Figure 4B).

The serum levels of T were significantly higher ($p=0.011$) at age 31, and FAI was significantly higher at age 31 ($p=0.002$) and 46 ($p=0.007$) in HDP women with srPCOS than in those without. After BMI adjustments, only the difference in FAI remained significant at both ages 31 ($P=0.012$) and 46 ($p=0.037$, Table 1B).

Association of hyperandrogenemia with HDP in the whole population

In the whole population, elevated T levels did not associate with HDP risk. The levels of FAI were significantly higher at ages 31 ($4.53 [2.62; 7.63]$ vs. $3.76 [2.43; 5.72]$, $p=0.009$) and 46 ($1.72 [1.25; 2.46]$ vs. $1.52 [1.06; 2.16]$, $p<0.001$) in women with HDP compared with women without HDP and the significance remained at age 46 after adjustment for BMI ($p=0.042$).

DISCUSSION

Main findings

This large follow-up cohort study indicates that the increased risk of HDP in srPCOS can mostly be attributed to overweight or obesity, but not to PCOS as such. More specifically, our study revealed also that besides overweight and obesity, weight gain from adolescence until the end of reproductive life was the most significant parameter associated with HDP both in srPCOS women and in non-PCOS women. Lastly, we could not confirm any significant association of hyperandrogenemia with the development of HDP.

Interpretation

In the whole group of women with srPCOS, the risk of HDP was significantly increased and in overweight/obese women with srPCOS the risk was more than three-fold compared to normal weight non-PCOS women. These findings are in line with the conclusions of recent meta-analyses, suggesting that women with PCOS have an increased risk of developing HDP.^{3,15-17} However, the difference disappeared in the whole population after adjustment for BMI and remained significant only when comparing the obese srPCOS women with the obese non-PCOS women. Moreover, the prevalence of HDP was not increased among normal weight srPCOS women compared to their normal weight non-PCOS counterparts. Similar results were found in the non-PCOS groups suggesting that the risk of HDP is mostly attributable to overweight/obesity, in line with some earlier data.^{30,31} The divergent conclusions of the aforementioned meta-analyses^{3,15-17} could be explained by the fact that the majority of the eligible studies either did not take into account confounding factors such as BMI or were of retrospective study design and included a relatively small sample size. Only few previous studies have addressed the possible interaction of BMI with PCOS regarding the risk of HDP. Lonnebotn et al also reported an increased risk of HDP in obese but not in normal weight or overweight women with PCOS.³² However, that study also reported increased risk in underweight (BMI <18.5 kg/m²) women, whereas there were no underweight women with both

srPCOS and HDP in our study. All in all, the results of the present study indicate that the increased risk of HDP in srPCOS can mostly be attributed to overweight/obesity.

This is an observational follow-up study and the main purpose was not to clarify the mechanism implicated in weight gain during pregnancy or their relationship with the development of HDP, but to investigate reproductive lifetime prevalence of HDP both in women with srPCOS and in control women. However, our results raise interesting hypotheses regarding possible mechanisms which can be discussed here. Maternal overweight/obesity before pregnancy and weight gain during pregnancy have been independently associated with an increased risk of HDP, compared with women with lower pre-pregnancy BMI and lower weight gain during pregnancy.^{33,34} One possible explanation for this association is the inflammatory changes leading to increased oxidative stress which further may result in endothelial dysfunction, maternal organ hypoperfusion and finally HDP.³⁴ The possible bias in maternal weight gain during pregnancy and the connection with HDP is the difficulty to differentiate between increase in adiposity and edema.

To the best of our knowledge, this is the first long-term follow-up study investigating the association of lifelong change of weight or of abdominal obesity (defined by waist circumference) with the risk of HDP. In this study, both srPCOS and non-PCOS women with HDP experienced a significantly greater increase in weight from adolescence to late adulthood compared with their counterparts without HDP. Although these results do not allow us to draw conclusions on causality, they suggest that normal weight prior to pregnancy may be at least in some extent protective against HDP. However, further intervention studies are needed to clarify this finding.

In the whole study population, the risk of HDP was not significantly increased in the group of women with elevated serum T levels compared with controls. Of note, FAI levels (with a trend for T levels) remained significantly associated with HDP regardless of BMI only

in the PCOS women. Some previous studies, but not all,²¹ have suggested that the hyperandrogenic PCOS phenotypes are associated with higher prevalence of HDP, especially preeclampsia, compared to normoandrogenic phenotypes.^{35,36} Hyperandrogenemia has been associated with preeclampsia also in the absence of PCOS.^{37,38} A possible explanation may be that placental aromatase (the enzyme responsible for the conversion of androgens to estrogens) is deficient in placental ischemia and preeclamptic pregnancy,³⁹ thus explaining the observed elevation of maternal androgens during preeclampsia. Elevated androgens have also been postulated to play an important role in the etiology of preeclampsia, although the mechanism is not clear.⁴⁰ In the present study, the lack of association between T and HDP in the whole study population may be partly due to the fact that we were not able to differentiate the diagnosis of preeclampsia from the other causes of HDP. Based on these and earlier results, the role of hyperandrogenemia in the pathogenesis of HDP remains therefore under debate and needs further research to be clarified.

Strengths of the Study

The main strength of this study is the prospective population-based cohort design with the longest follow-up time compared with previous studies. The participation rates for the clinical examinations and questionnaires at ages 31 and 46 were remarkably high and anthropometric parameters were mostly clinically measured. By taking advantage of FMBR, HDR and the questionnaire data we were able to have an accurate estimate of HDP in our study population. Moreover, careful adjustments for possible confounders allowed us to identify the respective effects of srPCOS and other risk factors on risk of HDP during reproductive life.

Limitations of the Study

A potential limitation of our study is that documenting symptoms of PCOS at age 31 and PCOS

313 diagnosis at age 46 was based on questionnaires. Due to the identification method of the PCOS
314 cases in the present study, the women most likely represent with milder hormonal and
315 metabolic profile than the women diagnosed with PCOS attending clinics. This study therefore
316 may, if anything, have underestimated the differences between PCOS and HDP. Hirsutism
317 might be over-reported by self-estimation and would have been better evaluated by clinical
318 examination and use of the Ferriman Gallwey semi-quantitative scoring system. Ovarian
319 ultrasonography was not available to aid the diagnosis of PCOS. However, this definition is
320 consistent with both the National Institutes of Health and the Rotterdam criteria for diagnosis
321 of PCOS.^{4,27} Moreover, in this same study population, we have previously shown that co-
322 existence of self-reported oligo-amenorrhea and hirsutism reliably identifies women with the
323 typical endocrine, metabolic and psychological profiles of PCOS and that the women with both
324 oligo-amenorrhea and hirsutism (who are considered as having srPCOS in the present study)
325 had the highest testosterone levels when compared to controls.^{27,28,41} The diagnoses of non-
326 classical congenital adrenal hyperplasia (CAH) were not performed. However, this feature has
327 most probably not biased the results, as the non-classical CAH is an extremely rare entity in
328 Finland with an incidence of 1:150 000.⁴²

329 It was not possible to differentiate between the diagnosis of chronic hypertension,
330 PIH and preeclampsia, as all these conditions were included as one identity into the HDP
331 diagnosis. We excluded from the analyses 522 women with only one source of diagnosis, which
332 may result in underestimation of the incidence of HDP. Finally, even though BMI values were
333 collected at ages 31 and 46 from clinical examination and postal questionnaire, BMI data at the
334 beginning of pregnancy or the data on weight gain during pregnancy were not available.
335 Women with at least one pregnancy were included in this study but the number of pregnancies
336 or recurrent HDP or non-recurrent HDP for each woman was not taken into account. However,
337 the aim of the present study was not to investigate weight gain during pregnancy but to

investigate reproductive lifetime prevalence of HDP both in women with srPCOS and in control women, independently of the age at pregnancy or the number of pregnancies.

Also, some women may have had their children before the age cut-off of 31 years.

In the present population, the mean age for the first delivery was 26.1 years.

Perspectives

The increased risk of HDP in srPCOS can mostly be attributed to overweight/obesity and weight gain through life. Importantly, normal weight women with srPCOS were not at increased risk for developing HDP. The present results suggest that maintaining normal weight may protect from HDP. However, the design of our study does not allow us to draw final conclusions on causality or mechanism and further intervention studies are needed to clarify this finding. The role of hyperandrogenemia in the pathophysiological process of HDP could not be confirmed, and remains debatable, with further research being needed to better identify its role.

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368

369 **Supporting Information**

370 Additional Supporting Information may be found in the online version of this article (please
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496 NOVELTY AND SIGNIFICANCE

497 What Is New?

- 498 • Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 5 to 15% of fer-
499 tile-aged women. Hyperandrogenemia and obesity (specifically abdominal obesity) are
500 common features of the syndrome and both contribute to the clinical and biochemical
501 derangements.
- 502 • Whether PCOS women have an increased risk of hypertensive disorders of pregnancy
503 (HDP) is still controversial. Moreover, it is still unclear which factors related to PCOS
504 associate with HDP. In this study we investigated whether women with self-reported
505 PCOS (srPCOS) have an increased prevalence of HDP during their reproductive life
506 and we aimed to clarify the impact of obesity and hyperandrogenemia on the develop-
507 ment of HDP. Specific interest was focused on weight gain from adolescence to adult-
508 hood in terms of the emergence of HDP both in PCOS and in non-PCOS women.
- 509 • To our knowledge, this is the first long-term, follow-up study exploring the association
510 of lifelong increase of weight or waist circumference with the risk of HDP. Both srP-
511 COS and non-PCOS women with HDP experienced a significantly greater increase in
512 weight from adolescence to late adulthood compared with their counterparts without
513 HDP. The increase in waist circumference between ages 31 and 46 was associated with
514 HDP, but not with PCOS status.

515 What Is Relevant?

- 516 • Our results highlight the existence of a complex synergistic interrelationship between
517 PCOS, obesity and weight gain and the risk of developing HDP. However, the design

of our study does not allow us to draw final conclusions on causality or mechanism and further intervention studies are needed to clarify this finding.

Summary

- The data of this study demonstrate that obesity and weight gain from adolescence to age 46, but not srPCOS or hyperandrogenemia, are associated with an increased risk of HDP.

Figure legends

Figure 1. Flowchart of the study population. Data on pregnancies, deliveries and HDP until the end of 2013 was obtained from the questionnaire at age 46, the Finnish Medical Birth Register (FMBR) and the Hospital Discharge Register (HDR).

FMBR: Finnish Medical Birth Register; H: hirsutism; HDP: hypertensive disorder of pregnancy; HDR: hospital discharge register; OA: oligo-amenorrhea; srPCOS: self-reported polycystic ovary syndrome.

Figure 2. Odds ratios (ORs) for hypertensive disorders of pregnancy (HDPs) in women with self-reported PCOS (srPCOS). The analyses were done using binary logistic regression. Results were adjusted model 1 (1) for consumption of alcohol, smoking and education or model 2 (2) for consumption of alcohol, smoking, education and BMI at age 31.

BMI: Body mass index; CI: confidence interval; HDP: hypertensive disorder of pregnancy; srPCOS: self-reported PCOS; T: total testosterone.

Figure 3. Odds ratios (ORs) for hypertensive disorders of pregnancy in women without PCOS (non-PCOS women). The analyses were done using binary logistic regression. Results were adjusted model 1 (1) for consumption of alcohol, smoking and education or model 2 (2) for consumption of alcohol, smoking, education and BMI at age 31.

BMI: Body mass index; CI: confidence interval; HDP: hypertensive disorder of pregnancy; srPCOS: self-reported PCOS; T: total testosterone.

Figure 4A. Body mass index (BMI) values during life in women with srPCOS and non-PCOS women according to their HDP status.

Figure 4B. Waist circumference (cm) values between ages 31 and 46 in women with srPCOS

and non-PCOS women according to their HDP status. BMI and waist circumference values are expressed as medians at each time point (14, 31 and 46 years). Pairwise comparisons at ages 14, 31 and 46 in each study group was analyzed using the Mann-Whitney U test or t-test. Only the P-values for comparison with non-PCOS women without HDP at the same age are presented here. The P-values for comparisons between the other groups are presented in the text and in Table 1.

Figures 4A and B: * $p < 0.05$, † $p < 0.01$ and ‡ $p < 0.001$.

BMI: body mass index; HDP: hypertensive disorder of pregnancy; srPCOS: self-reported polycystic ovary syndrome.

573 **Table 1A.** Clinical characteristics of non-PCOS women and women with srPCOS, sorted according to their status of hypertensive disorder of
574 pregnancy.

575 Clinical	Non-PCOS	Non-PCOS	P-value*	srPCOS	P-value†	srPCOS	P-value‡	P-value§	P-value
576 Characteristics	without HDP	with HDP		without HDP		with HDP			
577	(n=521-1039§)	(n=85-160§)		(n=88-147§)		(n=21-35§)			
578 BMI (kg/m ²) 14 yr	18.7 (17.4; 20.2)	19.2 (17.9; 20.9)	.011	19.3 (18.0; 20.6)	.006	20.4 (18.6; 22.8)	<.001	.022	.018
579									
580 BMI (kg/m ²) 31 yr	22.6 (20.7; 24.9)	24.0 (21.8; 26.7)	<.001	24.1 (21.5; 27.2)	<.001	28.0 (22.4; 33.9)	<.001	.001	<.001
581									
582 BMI (kg/m ²) 46 yr	25.2 (22.6; 28.6)	26.9 (23.9; 30.5)	<.001	26.2 (23.7; 30.3)	.002	31.4 (26.1; 37.3)	<.001 ^t	<.001 ^t	.003 ^t
583									
584 Waist (cm) 31 yr	76.0 (70.0; 83.0)	78.0 (72.5; 85.3)	.005	81.0 (71.0; 92.0)	<.001	83.5 (75.0; 98.0)	<.001	.113	.021
585									
586 Waist (cm) 46 yr	83.5 (77.0; 93.5)	90.0 (79.5; 99.0)	<.001	86.5 (79.5; 97.8)	.020	94.5 (83.6; 115.1)	.001 ^t	.003 ^t	.011 ^t
587									
588 Change in BMI									
589 (kg/m ²) 14-31 yr	3.83 (2.27; 5.74)	4.89 (3.21; 7.57)	<.001	4.59 (2.40; 7.54)	.009	5.94 (3.69; 11.1)	<.001	.015	.033
590									
591 Change in BMI									
592 (kg/m ²) 31-46 yr	2.40 (0.81; 4.19)	2.65 (0.97; 5.61)	.068	2.19 (0.71; 4.60)	.745	2.96 (0.14; 4.40)	.895	.801	.479
593									
594 Change in 14-46 yr									
595 BMI (kg/m ²)	6.33 (3.90; 9.33)	7.54 (5.32; 11.62)	<.001	7.21 (4.16; 10.5)	.151	9.82 (6.23; 14.6)	<.001 ^t	.001 ^t	.073 ^t
596									
597 Change in waist									
598 (cm) 31-46 yr	8.30 (2.60; 14.0)	8.80 (3.00; 16.0)	.352	8.00 (3.00; 15.0)	.692	11.0 (5.00; 19.9)	.203 ^t	.247 ^t	.691 ^t
599									

Table 1B. Clinical characteristics of non-PCOS women and women with srPCOS, sorted according to their status of hypertensive disorder of pregnancy.

Clinical Characteristics	Non-PCOS without HDP (n=521-1039 [§])	Non-PCOS with HDP (n=85-160 [§])	P-value ^{*,#}	srPCOS without HDP (n=88-147 [§])	P-value ^{†,#}	srPCOS with HDP (n=21-35 [§])	P-value ^{‡,#}	P-value ^{§,#}	P-value ^{,#}
Total testosterone (nmol/l) 31 yr	1.80 (1.40; 2.30)	1.90 (1.40; 2.40)	.527 .979 [#]	2.00 (1.70; 2.80)	<.001 .003 [#]	2.60 (1.95; 3.10)	.002 ^t .083 [#]	.284 ^t .558 [#]	.011 ^t .061 [#]
FAI 31 yr	3.75 (2.57; 5.44)	4.41 (2.67; 6.00)	.097 .985 [#]	5.08 (3.14; 7.64)	.002 .372 [#]	10.31 (4.55; 13.31)	.001 ^t .013 [#]	.015 ^t .189 [#]	.002 ^t .012 [#]
Total testosterone (nmol/l) 46 yr	0.82 (0.62; 1.05)	0.80 (0.61; 1.07)	.963 .664 [#]	0.86 (0.68; 1.05)	.277 .236 [#]	0.97 (0.72; 1.13)	.092 ^t .066 [#]	.315 ^t .232 [#]	.113 ^t .063 [#]
FAI 46 yr	1.53 (1.06; 2.22)	1.63 (1.17; 2.24)	.324 .727 [#]	1.62 (1.29; 2.25)	.064 .347 [#]	2.17 (1.43; 3.41)	.001 .037 [#]	.020 .176 [#]	.007 .037 [#]

Data is expressed as medians (interquartile ranges). The significance test used was Mann-Whitney U test, when a rule |skewness/standard error of skewness >1.96 was valid for the dependent variable, otherwise t-test was used (^t-sign). ^{*}P-value: non-PCOS with HDP compared to non-PCOS without HDP. [†]P-value: srPCOS without HDP compared to non-PCOS without HDP. [‡]P-value: srPCOS with HDP compared to non-PCOS without HDP. [§]P-value srPCOS with HDP compared to srPCOS without HDP. ^{||}P-value: srPCOS with HDP compared to non-PCOS with HDP. [§]The

623 numbers of women in separate analyses varies due to non-response to some items. [#]The results in table 1B were adjusted for BMI at age 31 or 46
624 using univariate general linear modelling (ANCOVA). BMI: body mass index; FAI: free androgen index; HDP: hypertensive disorder of pregnancy;
625 srPCOS: self-reported polycystic ovary syndrome; SHBG: sex hormone binding globulin.

626

Age 31: The odds ratios with 95% CIs for HDP

Age 46: The odds ratios with 95% CIs for HDP

Age 31: The odds ratios with 95% CIs for HDP

Age 46: The odds ratios with 95% CIs for HDP

4A

4B

ONLINE SUPPLEMENT

Association of self-reported polycystic ovary syndrome, obesity and weight gain from adolescence to adulthood with hypertensive disorders of pregnancy -a community-based approach

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Table S1. Body mass index (BMI) z-scores of non-PCOS women and women with srPCOS, sorted according to their status of hypertensive disorder of pregnancy.

Clinical	Non-PCOS	Non-PCOS	P-value*	srPCOS	P-value†	srPCOS	P-value‡	P-value§	P-value
Characteristics	without HDP	with HDP		without HDP		with HDP			
	(n=521-1039 [#])	(n=85-160 [#])		(n=88-147 [#])		(n=21-35 [#])			
zBMI 14 yr	-0.44 (-1.01; 0.10)	-0.24 (-0.79; 0.33)	.007 ^t	-0.20 (-0.73; 0.26)	.005 ^t	0.166 (0.48; 0.85)	<.001 ^t	.010 ^t	.012 ^t
zBMI 31 yr	-0.30 (-0.72; 0.22)	0.00 (-0.47; 0.62)	<.001	0.04 (-0.55; 0.72)	<.001	0.89 (-0.35; 2.22)	<.001	.001	<.001
zBMI 46 yr	-0.26 (-0.74; 0.39)	0.07 (-0.49; 0.73)	<.001	-0.07 (-0.53; 0.70)	.002	0.91 (-0.05; 1.99)	<.001 ^t	<.001 ^t	.002 ^t
Change in zBMI 14-31 yr	0.22 (-0.27; 0.77)	0.40 (-0.04; 1.16)	.005 ^t	0.33 (-0.29; 1.00)	.224	0.69 (0.22; 1.39)	<.001	.023	.075
Change in zBMI 31-46 yr	0.01 (-0.29; 0.33)	0.02 (-0.36; 0.48)	.281 ^t	-0.02 (-0.38; 0.32)	.150 ^t	-0.10 (-0.53; 2.25)	.159	.511	.127
Change in zBMI 14-46 yr	0.30 (-0.32; 0.92)	0.54 (-0.10; 1.26)	.005 ^t	0.24 (-0.24; 1.01)	.954	0.94 (0.01; 1.45)	.011 ^t	.023 ^t	.337 ^t

Data is expressed as BMI z-scores. The significance test used was Mann-Whitney U test, when a rule $|\text{skewness}/\text{standard error of skewness}| > 1.96$ was valid for the dependent variable, otherwise t-test was used (^t-sign). *P-value: non-PCOS with HDP compared to non-PCOS without HDP. †P-value: srPCOS without HDP compared to non-PCOS without HDP. ‡P-value: srPCOS with HDP compared to non-PCOS without HDP. §P-value srPCOS with HDP compared to srPCOS without HDP. ||P-value: srPCOS with HDP compared to non-PCOS with HDP. [#]The numbers of women in separate analyses varies due to non-response to some items. BMI: body mass index; HDP: hypertensive disorder of pregnancy; srPCOS: self-reported polycystic ovary syndrome.

Figure S1

Flowchart of the diagnosis of hypertensive disorder of pregnancy (HDP).

Data on HDP until the end of 2013 was combined from the questionnaire at age 46, the Finnish Medical Birth Register (FMBR) and the Hospital Discharge Register (HDR). The diagnosis for the final study population was assessed if at least two out of three sources was validated.

Data on women's pregnancies and deliveries up until the end of 2013 (at age 46) was obtained from the FMBR. The FMBR, active since 1987, is currently run by the National Institute for Health and Welfare. For each delivery in Finland, a structured form for FMBR is completed by the delivery hospital, including demographic and health data of the mother, the course and complications of the pregnancy (including HDP diagnosis) and delivery, and the perinatal health of the newborn up to the age of seven days. The FMBR is supplemented with data compiled by the Population Register Centre on live births and by Statistics Finland on stillbirths and deaths during the first week of life. After these additions, the registration of birth is 100%.

The HDR was checked for the data available (years 1972-2017) and the ICD9 and

ICD10 diagnoses (ICD10: O11*, O12*, O13*; O14*, ICD9: 6423, 6424, 6425, 6426, 6427) diagnostic codes for HDPs were identified.

In the NFBC1966 46-year postal questionnaire questions about HPD (chronic hypertension, PIH and preeclampsia) were asked as follows: If you have been pregnant, have you been diagnosed with high blood pressure during pregnancy (including preexisting chronic hypertension and PIH) or hypertension and proteinuria (=preeclampsia)? Pregnancy induced hypertension was defined as blood pressure greater than 140/90 mmHg after the 20th pregnancy week without proteinuria. Preeclampsia was defined as blood pressure greater than 140/90 mmHg after 20th pregnancy week with proteinuria (≥ 0.3 grams or a dipstick reading of 1+ or greater). Blood pressure was measured with a calibrated device and at least two separate measurements were performed at least four to six hours apart.²⁵

According to the questionnaire, 665 women were diagnosed with HDP by age 46. Of those women, 358 were not given a formal diagnosis of HDP according to the HDR or the FMBR. Their medical records were checked, and for 51 of those 358, the diagnosis of HDP was confirmed in the patients' records according to the ICD9 and ICD10 diagnoses (ICD10: O11*, O12*, O13*; O14*, ICD9: 6423, 6424, 6425, 6426, 6427). The remaining women whose diagnosis could not be confirmed were excluded from further analyses (n=307). Therefore, virtually all the women with only the diagnosis of essential hypertension were excluded from the analyses. After exclusion of the women with a diagnosis of HDP from only one source (n=522), the final study group therefore comprised 408 women with a confirmed diagnosis of HDP.

The women without diagnosis of HDP from any of these three sources were considered as control women (n=3373).

Laboratory methods

At age 31, SHBG was assayed as previously described ²⁸ and at age 46 by chemiluminometric immunoassay (Immulite 2000, Siemens Healthcare, Lanberis, UK). The intra-assay CVs of the method were 4.5% and 9.7% for concentrations 5.6 and 89.3 nmol/l and the inter-assay CVs 4.1% and 5.3% for concentrations 8.4 and 38.5 nmol/l, respectively. As the SHBG analysis method changed, the SHBG values from age 31 were transformed to be comparable with the SHBG values analyzed at age 46 using a formula: $0.7615 \times 31\text{yr SHBG (old method)} + 0.7088$.

Serum samples for assay of total testosterone (T) at ages 31 and 46 were analyzed by using Agilent triple quadrupole 6410 liquid chromatography mass spectrometry LC-MS equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE, USA). A woman was considered as having an elevated T levels if T was over 2.3 nmol/l at age 31 (upper limit in our accredited laboratory in fertile age women) or over 1.7 nmol/l at age 46. The cut-off value for T at age 46 was determined according to the upper limit of 97.5% reference range in non-PCOS women in the study population. The free androgen index (FAI) was calculated by using the equation $100 \times T \text{ (nmol/l)} / \text{SHBG (nmol/l)}$.

Figure S2

Body mass index (BMI) z-scores during life in women with srPCOS and non-PCOS women according to their HDP status.

Pairwise comparisons at ages 14, 31 and 46 in each study group were analyzed using the Mann-Whitney *U*-test or *t*-test. Only the P-values for comparison with non-PCOS women without HDP at the same age are presented here. The P-values for comparisons between the other groups are presented in Table S1.

Figure S1

Flowchart of the HDP diagnosis. Data on HDP until the end of 2013 was combined from the questionnaire at age 46, the Finnish Medical Birth Register (FMBR) and the Hospital Discharge Register (HDR). The diagnosis for the final study population was assessed if at least two out of three sources was validated.

The HDR was checked for the data available (years 1972-2017) and the ICD-9 (642*) and ICD-10 (O10*, O11*, O12*, O13*, O14*) diagnostic codes for HDPs were identified.

Figure S2. Body mass index (BMI) z-scores during life in women with srPCOS and non-PCOS women according to their HDP status.

Pairwise comparisons at ages 14, 31 and 46 in each study group was analyzed using the Mann-Whitney U test or t-test. Only the P-values for comparison with non-PCOS women without HDP at the same age are presented here. The P-values for comparisons between the other groups are presented in Table S1.