ORIGINAL ARTICLE

Type 2 diabetes mellitus in women with polycystic ovary syndrome during a 24-year period: importance of obesity and abdominal fat distribution

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STUDY QUESTION: What are the predictive factors for later development of type 2 diabetes (T2DM) in women with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: Obesity and abdominal fat distribution in women with PCOS in the mid-fertile years were the major risk factors for T2DM development 24 years later when lifestyle factors were similar to controls.

WHAT IS KNOWN ALREADY: Women with PCOS have an increased prevalence of T2DM.

STUDY DESIGN, SIZE, DURATION: A longitudinal and cross-sectional study was performed. Women with PCOS were examined in 1992 and in 2016. Randomly selected, age-matched women from the general population served as controls.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women with PCOS ($n = 27$), attending an outpatient clinical at a tertiary care centre for infertility or hirsutism were diagnosed in 1992 (mean age 30 years) and re-examined in 2016 (mean age 52 years). Women from the World Health Organization MONItoring of trends and determinants for CArdiovascular disease (WHO MONICA-GOT) 2008, aged 38–68 years, served as controls ($n = 94$), and they were previously examined in 1995. At both at baseline and at follow-up, women had blood samples taken, underwent a clinical examination and completed structured questionnaires, and the women with PCOS also underwent a glucose clamp test at baseline.

MAIN RESULTS AND THE ROLE OF CHANCE: None of women with PCOS had T2DM at baseline. At the 24-year follow-up, 19% of women with PCOS had T2DM versus 1% of controls ($P < 0.01$). All women with PCOS who developed T2DM were obese and had waist-hip ratio (WHR) >0.85 at baseline. No difference was seen between women with PCOS and controls regarding use of high-fat diet, Mediterranean diet or amount of physical activity at follow-up at peri/postmenopausal age. However, women with PCOS had a lower usage of a high-sugar diet as compared to controls ($P = 0.01$). The mean increases in BMI and WHR per year were similar in women with PCOS and controls during the follow-up period.

LIMITATIONS, REASONS FOR CAUTION: The small sample size of women with PCOS and the fact that they were recruited due to infertility or hirsutism make generalization to women with milder forms of PCOS uncertain.

WIDER IMPLICATIONS OF THE FINDINGS: Obesity and abdominal fat distribution, but not hyperandrogenism *per se*, in women with PCOS in the mid-fertile years were the major risk factors for T2DM development 24 years later when peri/postmenopausal. Lifestyle factors were similar to controls at that time.

STUDY FUNDING/COMPETING INTEREST(S): The study was financed by grants from the Swedish state under the agreement between the Swedish government and the country councils, the ALF-agreement (ALFGBG-718611), the Gothenburg Medical Association GLS 694291 and 780821, the Swedish Heart Lung Foundation and Hjalmar Svensson Foundation. The authors have no conflict of interest.

Key words: polycystic ovary syndrome / type 2 diabetes mellitus / menopausal / obesity / diet

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WHAT DOES THIS MEAN FOR PATIENTS?

This study looked at the development of type 2 diabetes mellitus in women with and without PCOS. A group of women with PCOS were followed from the age of about 30 up to over 50 years of age. Women with PCOS developed type 2 diabetes more frequently than women without PCOS (19%, compared with 1% in controls), but all women who did so were heavily overweight. Furthermore, in these women, their fat was distributed mainly around the waist, even in young women.

These results show the importance of early diagnosis of PCOS, and that doctors should monitor weight and waist circumference in all women with PCOS to evaluate the risk for later type 2 diabetes development. Advice should be given to women diagnosed with PCOS to avoid weight gain and thereby decrease the risk for type 2 diabetes development.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 9–18%, depending on the diagnostic criteria used (March *et al.*, 2010). The syndrome is, in addition to gynecological and hyperandrogenic features, associated with obesity, dyslipidemia, hypertension and hyperinsulinemia. A meta-analysis from 2018 showed an increased risk for impaired glucose tolerance (IGT) and increased risk for type 2 diabetes mellitus (T2DM), with three times increased odds of IGT (Kakoly *et al.*, 2018). The risk for IGT was increased regardless of whether women with PCOS and controls were matched for BMI or not, while on the other hand, the increased risk for overt T2DM was not significant if the groups were matched for BMI (Kakoly *et al.*, 2018). Most of the studies included in the meta-analysis were cross-sectional and included mainly women around the age of 30 years and younger, and only two of the included studies examined women with a mean age above 50 years (Kakoly *et al.*, 2018). In another recent review, the comorbidities of women with PCOS with a mean age of 40 years and above were examined (Cooney and Dokras, 2018). The results from this review suggested that the risk of T2DM remained increased in peri- and postmenopausal women with PCOS, although the results were diverse if adjusted or matched for BMI (Cooney and Dokras, 2018).

The new guidelines for the management of women with PCOS, published in 2018, recommend that glycemic status should be assessed at time of diagnosis and thereafter every 1–3 years, depending on the considered risk, for early detection of IGT and/or T2DM (Teede *et al.*, 2018). It is important to study if development of T2DM can be predicted. We have conducted a 24-year follow-up of women with PCOS who are now at peri- and postmenopausal age. The aim was to investigate whether it was possible to predict the development of T2DM in women with PCOS using data from the women of mid-fertile age. The women with PCOS were also compared with age-matched controls regarding T2DM, physical activity, dietary habits and changes in BMI and waist-hip ratio (WHR) over time.

Materials and Methods

Women with PCOS

In 1992, 33 women aged 20–39 years, attending Sahlgrenska University Hospital for infertility or hirsutism, were diagnosed with PCOS according to the National Institutes of Health (NIH) criteria (Zawadzki and Dunaif, 1992). They were then invited and agreed to participate in a study regarding hormonal treatment for hirsutism or infertility, which included clinical examination, blood sampling and structured interviews before the intervention (Dahlgren *et al.*, 1998). Thirty-two of them finally attended the initial study (mean age 29 years, age range 19–38 years) and were allocated to two antiandrogen regimens: 28 women completed the trial. If hirsutism was the main complaint, the treatment was 50 mg ethinyloestradiol + 100 mg cyproterone acetate (EO-CA). If infertility was the main complaint, the treatment was the GnRH analogue goserelin, 3.6 mg sc every 28th day. A total of 20 women were treated with EO-CA cyclically for 6 months and 8 women were treated with goserelin for 6 months. As part of routine care, the women also received advice about lifestyle.

A follow-up study was carried out in 2016 with similar examinations. Five of the 33 women with PCOS were deceased and one declined participation. Twenty-seven women with PCOS were included, with an age range of 42–63 years. Of these, 25 participated in all parts of the follow-up study and two completed the interviews only. Thirty-seven percent of the women with PCOS were postmenopausal at the follow-up examination (Forslund *et al.*, 2019).

The protocols from 1992 were reviewed to establish the PCOS phenotype. All 27 women with PCOS fulfilled both the clinical hyperandrogenism and the oligo-/amenorrhea criteria. Thus, they also fulfilled the previously used NIH criteria and thereby the Rotterdam criteria, including the updated definition (Fauser *et al.*, 2004; Teede *et al.*, 2018).

Controls

A randomly selected population sample was examined in 1994–1995 (25–64 years, *n* = 872 women) as part of the World Health Organization (WHO) study, MONItoring of trends and determinants for CArdiovascular disease (MONICA) 1995, Gothenburg, Sweden (Wilhelmsen *et al.*, 1997). They were re-examined in 2008, with a participation rate of 65% (Trimpou *et al.*, 2012). Out of 317 women in the age group 39–78 years, 95 women were selected as an age-matched control group using a group matching model. A physical examination and structured interviews using standardized questionnaires were performed in 2008. The data was evaluated. None of the controls had a history of irregular periods. One woman was excluded due to a testosterone level above the reference interval at that time, leaving a final control group of 94 women without biochemical hyperandrogenism, age range 38–68 years. The women in the control group came from the same geographic area as the women with PCOS. Fifty-seven percent were postmenopausal (Forslund *et al.*, 2019).

Anthropometry

Height was measured to the nearest 0.5 cm. Body weight was measured in underwear to the nearest 0.5 kg. For the two women with

PCOS who did not participate in the clinical investigation, self-reported weight and height were used. BMI was calculated as body weight divided by height squared (kg/m^2). Waist and hip circumferences were measured to the nearest cm in the standing position over the umbilicus and the maximum circumference over the buttocks, respectively, and the WHR was calculated.

At baseline, total body potassium was determined in a whole-body counter detecting natural ^{40}K (Nuclear Enterprise, Edinburgh, UK) and expressed in mmol. Lean body mass (LBM) was then estimated, as previously described (Dahlgren et al., 1998).

Structured medical history

Data was collected concerning self-reported current and previous diseases, including T2DM. The T2DM diagnosis was also verified in medical records and all women with T2DM used antiglycemic drugs. History involving smoking, parity and marital status was also registered. Current medications, including metformin and insulin, were recorded.

Structured questionnaires regarding physical activity level during leisure time and at work were used in the follow-up examination. Physical activity at work was graded as predominantly sedentary, i.e. included some walking and standing but no stairs or heavy lifting, involved walking including stairs or walking uphill and/or lifting heavy objects or corresponded to heavy physical labor. Physical activity during leisure time was graded as predominantly sedentary, i.e. reading or watching television; included moderate activity such as walking, riding a bicycle and/or light garden work for at least 4 h per week; involved regular exercise such as running, swimming, tennis and heavy gardening for at least 2 to 3 h per week; and was defined as regular athletic training and/or participation in competitive sports several times per week. The grading was based on questionnaires previously used to assess the relation between physical activity and risk of myocardial infarction (Saltin and Grimby, 1968; Wilhelmsen et al., 1976). For analysis, the four possible levels of activity were grouped into a binary variable— 'sedentary' (grades 1 and 2) or 'active' (grades 3 and 4) lifestyle, during leisure and work, respectively.

A food frequency questionnaire (FFQ) was used to evaluate dietary habits. Questions on how often the women consumed 83 different groups of food items were asked for, with nine possible answers ranging from score 1 (never) to score 9 (four times per day or more). For comparison and statistical analysis, the scores were recoded into frequencies per week: if a food item were never used, a few times per year or 1–3 times per month, it was calculated as 0 times/week. The other possible answers were calculated as: 1 time/week = 1 time/week, 2–3 times/week = 2.5 times/week, 4–6 times/week = 5 times/week, 1 time/day = 7 times/week, 2–3 times/day = 17.5 times/week and 4 or more times/day = 30 times/week. A similar FFQ has been used and validated in the Northern Sweden Health and Disease Cohort (Johansson et al., 2002).

Three categories were created reflecting food with high fat content including food items with a high content of saturated fat such as butter, margarine, cheese, cream, French fries, pizza, sausage, hamburger, meatballs, liverwurst, bacon, crisps, chocolate, ice cream, cakes and pastry; high sugar content including food items such as buns, cookies, pastry, cakes, sweets, sweet beverages, sugar and jam; and Mediterranean diet including vegetables, fruit, berries, vegetable oils and fish.

Total scores based on frequencies for the different diets were then calculated with a higher score corresponding to a higher usage of that kind of food. Possible ranges for the different categories were high fat content—0–630, high sugar content—0–240 and Mediterranean diet—0–540.

Biochemistry

Baseline biochemistry for women with PCOS

Blood glucose was analysed using a glucose oxidase method (Kabi, Stockholm, Sweden), reference range 4.2–6.3 mmol/L. Plasma insulin was determined with a RIA method (Byc-Sangtec, Ditzenbach, Germany), reference level <20 mU/L. Serum total testosterone was determined by non-extraction competitive radioimmunoassay (Radioassay System Laboratories ^{125} IT; ICN Biochemicals Inc., Diagnostics Division, Costa Mesa, CA, USA), reference <3.0 nmol/L. Sex hormone-binding globulin (SHBG) was determined by an immunoradiometric assay (IRMA) (Farmos Group Ltd, Oulunsalo, Finland).

Follow-up examination for women with PCOS and controls

Plasma glucose concentrations were measured using an enzymatic hexokinase method for controls, GLU; Roche/Hitachi; Roche Diagnostics, GmbH, Mannheim, Germany with a 4% coefficient of variation (CV) at concentrations between 5 and 15 mmol/L, and for women with PCOS, GLUC3; Roche/Cobas; Roche Diagnostics Scandinavia with a 3% CV at concentrations between 5 and 15 mmol/L.

Serum insulin concentrations for controls were measured with an immunometric two-step sandwich method and chemiluminescence technology (Insulin Elecsys, Roche Diagnostics GmbH, Mannheim, Germany) with a 10% CV at 6, 20 and 70 mU/L. For the women with PCOS, the analysis was similar, an electrochemiluminiscense imumuno assay (ECLIA) sandwich method (Insulin Elecsys, Roche Diagnostics Scandinavia, Angelholm, Sweden) with a 10% CV at 6, 20 and 180 mU/L.

Serum total testosterone in the PCOS group was determined by an electrochemiluminescent immunoassay with competitive analysis (ECLIA), (Cobas 8000 Roche Diagnostics Scandinavia AB, Angelholm, Sweden). The CV was 6% at 2.0 nmol/L. Serum total testosterone in the control group was determined by nonextraction competitive RIA (ICN Biochemicals, Inc. Diagnostics Division, Costa Mesa, CA, USA). The CV for total testosterone levels was 16.3% at 2.0 nmol/L.

SHBG in the PCOS group was analyzed using the sandwich principle (Cobas 8000 Roche Diagnostics Scandinavia AB, Angelholm, Sweden), and the CVs for SHBG were 7% at 40 nmol/L and 9% at 100 nmol/L. In the control group, SHBG was determined by IRMA (Orion Diagnostica Oy, Espoo, Finland), the CV for SHBG levels was 4.2% at 19.7 nmol/L and 6.3% at 76.3 nmol/L.

Free androgen index (FAI) was calculated as total testosterone/SHBG × 100. Insulin sensitivity was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as fasting insulin (μU/L) × fasting glucose (nmol/L)/22.5 (Matthews et al., 1985).

Glucose clamp technique

A hyperinsulinemic, euglycemic glucose clamp (DeFronzo et al., 1979) was used to estimate insulin sensitivity in women with PCOS at the

Table I Baseline data for women with polycystic ovary syndrome and those who developed, and did not develop, type 2 diabetes mellitus during the 24-year follow- up.

	Women with PCOS (n=27)		T2 dm (n=5)		Not T2DM (n=22)		
	mean (SD)	median (range)	mean (SD)	median (range)	mean (SD)	median (range)	P-value
Age (years)	29.5 ± 5.3	30 (20–39)	29.6 ± 6.5	30 (20–36)	29.5 ± 5.1	30 (20–39)	0.93
BMI (kg/m2)	27.3 ± 6.0	25.9 (19.0–41.4)	34.7 ± 4.5	32.3 (30.7–41.4)	25.6 ± 5.0	24.8 (19.0–39.6)	**<0.01**
Waist (cm)	88 ± 14	86 (64–125)	106 ± 12	106 (96–125)	84 ± 12	83 (64–106)	**<0.01**
WHR	0.83 ± 0.09	0.82 (0.73–1.11)	0.96 ± 0.10	0.96 (0.86–1.11)	0.80 ± 0.064	0.79 (0.73–1.00)	**<0.01**
P-glucose (mmol/L)	4.8 ± 1.0	4.7 (3.9–80)	5.3 ± 1.6	4.9 (3.9–8.0)	4.7 ± 0.8	4.6 (3.9–8.0)	0.23
S-insulin (mU/L)	14.3 ± 18.1	9.0 (4.2–97)	31.8 ± 36.5	17.0 (12.0–97.0)	9.9 ± 5.9	8.5 (4.2–27.0)	**<0.01**
HOMA-IR	3.5 ± 5.8	12.4 (1.3–28.5)	7.8 ± 5.3	10.6 (1.3 = 13.1)	15.0 ± 5.0	14.6 (7.4–28.6)	**<0.01**
GIR-LBM (mg/kg/min)	13.5 ± 5.8	12.4 (1.3–28.5)	7.8 ± 5.3	10.6 (1.3–13.1)	15.0 ± 5.0	14.6 (7.4–28.6)	0.02
S-testosterone (nmol/L)	1.86 ± 0.87	1.79 (0.24–3.94)	2.00 ± 1.49	1.67 (0.24–3.94)	1.83 ± 0.72	1.83 (0.73–3.85)	0.98
S-SHBG (nmol/L)	48.7 ± 43.4	33.0 (13.0–181.0)	45.3 ± 57.2	17.5 (15.0–131.0)	49.3 ± 42.0	33.0 (13.0–181.0)	0.23
FAI	6.8 ± 6.1	5.4 (0.2–26.3)	10.5 ± 11.2	8.0 (0.2–26.3)	6.1 ± 4.8	5.2 (0.5–18.2)	0.46

PCOS = polycystic ovary syndrome, T2DM = type 2 diabetes mellitus, WHR = waist hip ratio, HOMA-IR = homeostasis model assessment of insulin resistance,
GIR-LBM = glucose infusion rate with lean body mass, FAI = free androgen index, SHBG = sex hormone-binding globulin, P = plasma; S = serum.
P-value for the comparison between those who later developed T2DM and those that did not are shown. Significant P-values (<0.05) are marked as bold. Variables normally distributed were tested with the independent samples Student's
t-test, data that were not normally distributed were tested with the Mann–Whitney U test.
For insulin and HOMA-IR *n* = 25 for women with PCOS and *n* = 20 for not T2DM.
For GIR-LBM, *n* = 18 for not T2DM and *n* = 23 for women with PCOS.
For SHBG and FAI *n* = 25 for women with PCOS, *n* = 4 for T2DM and *n* = 21 for not T2DM.

Table II Number of women with PCOS and with or without T2DM at 24 years' follow-up, stratified according to normal weight/overweight (BMI < 30 kg/m^2) or obesity (BMI ≥ 30 kg/m^2) at baseline and WHR more or less than 0.85 at baseline, respectively.

	T2DM ($n = 5$)	Not T2DM ($n = 22$)
BMI <30 kg/m^2	0	20
BMI >30 kg/m^2	5	2
WHR ≤ 0.85	0	18
WHR >0.85	5	4

P-value <0.01 for both obesity and WHR >0.85.

baseline investigation (Krotkiewski et al., 2003). After an overnight fast, an indwelling catheter was inserted into an antecubital vein for glucose, insulin and potassium administration. A second catheter was placed into a hand vein of the contralateral arm for blood sampling. After 10 min infusion of a priming dose of insulin, a constant dose of insulin with an infusion rate of 0.08 IU/kg body weight/min was given, which corresponded to insulin levels of ~200 mU/L. Blood glucose levels were kept constant at 4.9–5.0 mmol/L by the continuous infusion of glucose. Potassium was infused at a rate of 50 ml/h. The clamp was performed for 2 h and the glucose disposal rate was calculated from the steady-state glucose infusion rate over the last 30 min. During this steady state, the glucose infused equals the glucose metabolized. The glucose disposal rate was expressed as mg/kg LBM/min, since the skeletal muscles are the major pools of glucose elimination in response to insulin.

Ethical approval

The study was approved by the Regional Ethical Review Board in Gothenburg (reg. no. 221-16, reg. no. 088-06 and reg. no. T282-11), and all the women gave their written informed consent.

Statistics

Mean value, median, SD and range were calculated with conventional methods. Intergroup comparisons of continuous variables that were normally distributed were calculated with the independent samples Student's t-test. Data that were not normally distributed were tested with the Mann–Whitney U test. Categorical comparisons were calculated using Fischer's exact t-test.

Odds ratios (ORs) were computed with a multiple variate logistic regression analysis to compare the relative impact of PCOS and BMI on T2DM diagnosis.

SPSS Statistics version 25 (IBM Corp., Released 2017, IBM SPSS Statistics for MAC, Armonk, NY, USA) was used for the analyses.

A post hoc power calculation was performed (G∗power) for the differences in prevalence of T2DM between women with PCOS and controls. With $\alpha = 0.05$ and the prevalence found in this study (19% versus 1%) this gave an achieved power of 0.91.

Results

Longitudinal changes within the PCOS group

Women with PCOS who 24 years later developed T2DM (19%) had higher mean values of BMI (35 kg/m^2 versus 26 kg/m^2), waist circumference (106 cm versus 84 cm), WHR (0.96 versus 0.80), S-insulin levels (32 mU versus 10 mU/L) and HOMA-IR (9.5 versus 2.0) at baseline than women who did not develop T2DM (Table I). All women with PCOS who developed T2DM were obese (BMI >30 kg/m^2) and had abdominal fat distribution with a WHR >0.85 at baseline, as shown in Table II. Out of the women with PCOS who were obese at baseline, 71% had converted to T2DM by follow-up and 56% of PCOS women who had WHR >0.85 had developed T2DM. There were no differences in development of T2DM between the initial PCOS intervention groups (EO-CA or GnRH analogue treatment in 1992) (data not shown).

The results from the glucose clamp test at baseline showed lower mean glucose infusion rates (8 compared to 15 mg/kg LBM/min) for those who 24 years later developed T2DM versus those who did not. However, the serum testosterone, SHBG or FAI did not differ between the groups with or without T2DM (Table I).

Table III Anthropometric data and characteristics of women with PCOS and controls at follow-up and at baseline 13–24 years earlier.

	PCOS $n = 27$*	Controls $n = 94$	P-value
Age (years)	52.4 ± 5.4	52.4 ± 6.3	0.68
Age baseline (years)	29.5 ± 5.3	39.7 ± 6.5	**<0.01**
Body weight (kg)	86.3 ± 21	70.1 ± 11	**<0.01**
Body weight baseline (kg)	77.9 ± 18.6	65.6 ± 10.2	**<0.01**
Delta weight/year (kg)	0.37 ± 0.59	0.35 ± 0.54	0.61
Height (cm)	168 ± 6	166 ± 6	0.13
BMI (kg/m^2)	30.7 ± 7.4	25.5 ± 3.9	**<0.01**
BMI baseline (kg/m^2)	27.3 ± 6.0	23.6 ± 3.3	**<0.01**
Delta BMI/year (kg/m^2)	0.15 ± 0.22	0.15 ± 0.20	0.64
Waist circumference (cm)	102 ± 18	87 ± 11	**<0.01**
Hip circumference (cm)	113 ± 15	104 ± 9	**<0.01**
WHR	0.90 ± 0.12	0.83 ± 0.06	**0.02**
WHR baseline	0.83 ± 0.09	0.79 ± 0.05	**0.02**
Delta WHR/year	0.003 ± 0.005	0.004 ± 0.004	0.49
Current smoker, n (%)	3 (11)	9 (10)	1.00
Never smoker, n (%)	12 (44)	49 (53)	0.51

Means ± SD are given. If not otherwise stated, data from the re-examinations are shown. Delta body weight/year, delta BMI/year and delta WHR/year denotes the change in body weight, BMI and WHR, respectively, per year of follow-up. Significant P-values (<0.05) are marked in bold. Intergroup comparisons of continuous variables that were normally distributed were tested with the independent samples Student's t-test. Data that were not normally distributed were tested with the Mann–Whitney U test. Categorical comparisons were calculated using Fischer's exact t-test.

*Except for waist, hip, WHR and delta WHR where $n = 25$.

The dietary habits and physical activity level during leisure or work did not differ between women with PCOS with or without T2DM at follow-up (data not shown).

Comparison between women with PCOS and controls

The mean age at follow-up was 52 years, with no difference between women with PCOS and controls. Since the examination at baseline occurred at a younger age for women with PCOS, there was a 10-year difference in mean age at baseline: 30 years for women with PCOS compared to 40 years for controls. No difference in smoking habit was observed (Table III).

At follow-up, the women with PCOS, in comparison to controls, had higher mean values of body weight (86 kg compared to 70 kg, respectively) and BMI (31 kg/m^2 compared to 26 kg/m^2, respectively). Since the durations from baseline to follow-up differed between the PCOS cases and the controls, the delta values (per year change) were evaluated. No differences in delta values were seen, with a BMI increase of 0.15 kg/m^2/year for both groups and delta WHR 0.003/year in PCOS and 0.004/year in controls (Table III). In spite of this, women with PCOS had a persistently higher body weight and WHR than controls at follow-up (Table III).

At baseline, none of the women with PCOS had diabetes mellitus, while in the control group one (1%) had type 1 diabetes mellitus and none had T2DM. At follow-up five (19%) of the women with PCOS had developed T2DM compared to one (1%) of the controls ($P < 0.01$). When comparing the women with and without PCOS who were obese at baseline, 71% of the obese PCOS women had converted to T2DM compared to 25% of obese controls (P-value 0.24).

No additional cases of T2DM based on elevated fasting plasma glucose were found. The use of insulin and metformin (irrespective of type of diabetes) is shown in Table IV. All women (with or without PCOS) who had T2DM diagnosis were obese at follow-up, and all women with PCOS and T2DM also had a WHR >0.85, whereas the one control with T2DM had a WHR <0.85.

Women with PCOS had a higher HOMA than controls (median 1.9 compared to 1.4; Table IV).

At follow-up, there was no difference between women with PCOS and controls when analyzing sedentary lifestyle during leisure time or at work, use of a Mediterranean diet or a diet with a high content of saturated fat. However, women with PCOS used a diet containing less sugar compared with controls ($P = 0.01$; Table IV).

At follow-up, there was no difference in levels of SHBG or FAI between PCOS and control groups, but there was a tendency towards higher levels of serum total testosterone among those with PCOS (median for S-testosterone for women with PCOS 0.83 nmol/L compared to 0.62 nmol/L in controls, $P = 0.06$; Table IV).

The unadjusted OR for T2DM was 21 times higher for women with PCOS compared to controls (Table IV).

Discussion

The main finding of the present study was that women with PCOS developed T2DM to a high extent (19%) during a 24-year follow-up period from their fertile years into the peri- and postmenopausal age. These women already had obesity, and especially abdominal fat distribution, at baseline in the mid-fertile age. Furthermore, they had a high HOMA index and low insulin sensitivity according to the hyperinsulinemic, euglycemic glucose clamp. Hence, the risk for T2DM in women with PCOS can be predicted, as we had hypothesized. Hyperandrogenicity *per se* was not attributable to T2DM in PCOS.

Women with PCOS had a higher BMI and WHR, but the increases in BMI and WHR per year were similar in controls and PCOS during the follow-up periods. Thus, the obesity and abdominal fat distribution was present at a relatively young age among women with PCOS and the increase was then in parallel in PCOS and controls.

The second hypothesis was that the development of T2DM in PCOS was independent of lifestyle factors. This could be verified as the degree of physical activity and dietary factors, at least at follow-up, were not different from controls. In fact, women with PCOS had a diet containing less sugar than controls.

The results regarding prevalence of T2DM in women with PCOS over the age of 40 years are conflicting. In another cohort of women with PCOS followed for a median of 13 years, there was no difference in hazard ratio for T2DM above 40 years, as compared to controls (Kazemi Jaliseh *et al.*, 2017). In our previous study of another cohort of women with PCOS and controls, where the women had a mean age of 72 years and similar BMI, the prevalence of T2DM was 22% for women with PCOS compared with 14% for controls (not statistically significant) (Schmidt *et al.*, 2011). The lack of significance might be due to the similar BMI between groups in that study and the fact that controls caught up in WHR during the 21-year follow-up period (Schmidt *et al.*, 2011). The menopause also influences metabolism, with increased insulin resistance and changes in lipid metabolism with increasing lipid levels (Mauvais-Jarvis *et al.*, 2013; Paschou and Papanas, 2019; Paschou *et al.*, 2019). In our study, the proportion of postmenopausal women was higher in the control group than in the PCOS group (Forslund et al., 2019), thus the difference could actually have been further accentuated if more women with PCOS had been postmenopausal.

In a prospective population-based birth-cohort study from Finland, women (aged 46 years) with a history of oligo-/amenorrhea and hirsutism had 2.5 times higher odds of T2DM compared with controls, but no case of T2DM was found in normal weight women with PCOS at this age (Ollila *et al.*, 2017). In the present study, where the women with PCOS were initially recruited from a hospital setting, only those with a BMI > 30 kg/m^2 (i.e. obese) at the initial assessment had developed T2DM at the 24-year follow-up, but this was also true for the single control who developed T2DM. The prevalence of T2DM in the control group was lower (1%) compared to the Finnish control group (4%), which could at least partly be explained by the lower mean BMI in the control group in the present study.

In this study, there was no difference in the baseline data regarding total S-testosterone, SHBG or FAI when comparing those who later developed T2DM with those who did not. None of the women with PCOS who were neither obese nor had an abdominal fat distribution at baseline but who developed obesity at follow-up, developed T2DM at perimenopausal age. Thus, abdominal obesity before mid-fertile age was a strong risk factor for development of T2DM in women with PCOS. This is in line with previous studies on the general population where overweight and obesity, especially when present at a young age, significantly increased the lifetime risk for T2DM (Narayan *et al.*, 2007).

Our results show that women with PCOS had a significantly higher BMI and WHR both at baseline and at follow-up, but that this

Table IV Prevalence of T2DM, use of antiglycemic drugs, lifestyle factors and biochemistry in women with PCOS and controls.

	PCOS n = 27		Controls n = 94		P-value
T2DM, n (%)	5 (19)		1 (1)		<**0.01**
Insulin treatment, n (%)	3 (11)		2 (2)		0.07
Metformin treatment, n (%)	3 (11)		1 (1)		**0.03**
Sedentary lifestyle, n (%)	18 (67)		57 (63)		0.82
Sedentary work, n (%)	23 (89)		79 (89)		1.00
	mean ± SD	median (range)	mean ± SD	median (range)	
High fat diet		44 (9–104)		45 (0–125)	0.57
High sugar diet		6 (1–36)		9 (0–56)	**0.01**
Mediterranean diet		72 (26–261)		79 (18–293)	0.47
P-glucose (mmol/L)	6.0 ± 1.7	5.4 (5–13)	5.0 ± 1.0	4.8 (4–13)	<**0.01**
S-insulin (mU/L)	14.1 ± 13.6	7.7 (3–52)	7.6 ± 5.7	6.4 (1–47)	0.07
HOMA-IR	4.5 ± 6.4	1.9 (0.8–29.6)	1.7 ± 1.5	1.4 (0.3–12.5)	**0.02**
S-SHBG (nmol/L)	66.1 ± 37.8	54.0 (25–178)	58.5 ± 25.7	57.1 (19.6–177.3)	0.70
S-testosterone (nmol/L)	1.01 ± 0.66	0.83 (0.40–2.70)	0.67 ± 0.27	0.62 (0.14–1.28)	0.06
FAI	2.2 ± 2.3	1.4 (0.3–10.8)	1.4 ± 1.0	1.1 (0.2–5.0)	0.40
OR of T2DM		unadjusted OR	CI (95%)		
	PCOS	21.14	2.35–190.14		
	BMI	1.45	1.17–1.80		

High scores at the FFQ correspond to higher usage of that kind of food, high fat diet—possible score range, 0–630; high sugar diet—possible score range, 0–240; Mediterranean diet—possible score range, 0–540.

Significant P-values (<0.05) are marked as bold. Categorical comparisons were calculated using Fischer's exact t-test. Intergroup comparisons of continuous variables that were normally distributed were tested with the independent samples Student's t-test. Data that were not normally distributed were tested with the Mann–Whitney U test. ORs were computed with a multiple variate logistic regression analysis, to compare the relative impact of PCOS and BMI on T2DM diagnosis at follow-up.

difference compared to controls occurred before mid-fertile age. During the years of follow-up (24 years for women with PCOS and 13 years for women with no PCOS), the average increases in BMI and WHR per year did not differ. This further emphasizes the need for early diagnosis and thereby the possibility to treat and start lifestyle interventions at an early age. The treatments (EO-CA or GnRH analogue) that women with PCOS received 24 years ago did not affect development of T2DM in spite of a worsening of the glucose disposal rate by EO-CA treatment (Dahlgren et al., 1998). However, even if the PCOS phenotype occurs soon after adolescence there is a delay of several years until it is properly diagnosed and thereby a delay in the prevention of obesity and subsequent T2DM development. A recent study showed that one-third of the women had to wait more than 2 years after initial contact with the healthcare system to obtain a PCOS diagnosis and nearly one-half had to see ≥3 different physicians before being diagnosed (Gibson-Helm et al., 2017).

All women with PCOS who later developed T2DM had reduced insulin sensitivity, as measured by a clamp test and expressed per kg LBM. The lean mass, mainly the skeletal muscle tissue, is the primary target for peripheral glucose uptake. At the same time as the women with PCOS in this study, in the same laboratory and with the same method, clamp studies were performed (by the coauthor K.L.W.) in obese and lean women with high and low WHR, respectively (Landin

et al., 1990). The mean glucose disposal rate was similar in abdominally obese women without PCOS in that study (11 mg/kg LBM/min) as in the women with PCOS with similar body composition of the present study. Women with peripheral fat distribution (WHR ≤ 0.85) irrespective of BMI had a higher glucose disposal rate (16 mg/kg LBM/min), similar to the non-diabetic women with PCOS in the present study (Landin et al., 1989). This emphasizes the importance of abdominal fat distribution for the deterioration of glucose metabolism.

We also compared some specific lifestyle factors between women with PCOS and controls at follow-up. No difference was seen regarding sedentary lifestyle, which supports previous findings from a younger cohort of women with PCOS with a mean age of 34 years (Moran et al., 2017), and also that of an older cohort with a mean age of 71 years (Schmidt et al., 2012). The dietary habits were also compared in the present study, and it was found that the women with PCOS had a diet with less sugar compared with the controls. No differences were seen regarding fat intake or use of a Mediterranean diet. Previous studies have shown a tendency towards a healthier diet, including higher use of a Mediterranean diet, in women with PCOS (Moran et al., 2015; Moran et al., 2017). This might be due to previous dietary advice, since lifestyle intervention is the first line of treatment for PCOS, but also to the fact that T2DM is more common among women with PCOS and they would then get advice to change their diet.

A limitation of this study was the small sample size in the PCOS group and that the women with PCOS were recruited because of infertility or hirsutism, which makes generalization to women with milder forms of PCOS uncertain. Another limitation is that the testosterone levels were analyzed with an immunoassay method, which is the available method in our laboratory but this is not considered the gold standard. It has previously been shown that mass spectrometry only minimally improves discrimination of women with and without PCOS compared with a direct testosterone immunoassay (Handelsman *et al.*, 2017). The strengths were the establishment of the PCOS diagnosis during the reproductive years and the long follow-up time of 24 years for the women with PCOS. In addition, all women came from the same geographical area. Furthermore, a random population sample of women used as controls is considered to be the best group for valid comparisons. However, this made it impossible to match for BMI owing to the few obese women in the general population at that time.

Clinical implications of this study include the importance of early diagnosis of PCOS and estimating BMI and WHR to evaluate the risk for later T2DM development. The new guidelines for PCOS recommend screening for glycemic status every 1–3 years and in high risk women with PCOS, including women with a BMI >25 kg/m^2, an oral glucose tolerance test is recommended (Teede *et al.*, 2018). Measurement of WHR can help to further evaluate the individual's risk for future IGT and T2DM and help the healthcare system to enhance lifestyle interventions at an early stage in those with the highest risks.

In conclusion, abdominal obesity in PCOS in women of mid-fertile age, but not the hyperandrogenism *per se*, was the major risk factor for T2DM development 24 years later at peri-/postmenopausal age, when lifestyle factors were similar to controls.

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Authors' roles

M.F., K.L.W., M.B., J.S. and E.D. designed the study. M.F., E.D., K.L.W. and P.T. examined the women. M.F. analyzed the data and made the first draft of the article. M.F., K.L.W., M.B., J.S. and E.D. all contributed to the interpretation of data. All authors made contributions to drafting and revising the article and all authors approved final version of the article.

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Conflict of interest

The authors have no conflict of interest to declare.

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