human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Low fertility and the risk of type 2 diabetes in women

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BACKGROUND: Fertility problems are frequently followed by early menopause, and early menopause has been associated with increased risk of type 2 diabetes (T2D). Thus far, it is unknown whether low fertility is independently associated with future T2D risk.

METHODS: We assessed the association between measures of low fertility and T2D in the Prospect-European Prospective Investigation into Cancer and Nutrition (EPIC) cohort of 17 357 Dutch women, aged 49–70 years at baseline using Cox proportional hazards models, adjusted for various confounders. To investigate whether BMI and waist circumference influence the observed associations, analyses were additionally adjusted for these variables.

RESULTS: At baseline, 332 women had T2D. During a mean follow-up of 9.1 ± 3.6 years, 535 T2D cases occurred. Out of 15 707 Prospect-EPIC women who wanted to get pregnant, 1940 consulted a physician for fertility problems and 700 remained childless. No relation was found between consulting a physician for fertility problems or nulliparity and T2D risk. Of all women who wanted to get pregnant, 3946 (25.1%) had one or more miscarriages, with an average of 1.4 (± 0.9) miscarriages and a maximum of 10 miscarriages. Women who had one or more miscarriage showed the same risk for T2D as women who had no miscarriage. Also, none of the other measures of low fertility were associated with increased risk for T2D.

CONCLUSIONS: Generally, measures of low fertility were not independently associated with a risk of T2D in a cohort of 17 357 Dutch women.

Key words: type 2 diabetes / low fertility / infertility / miscarriages / irregular menstrual cycle

Introduction

Compared with all other populations with a modern lifestyle, the age-adjusted prevalence of type 2 diabetes (T2D) in populations of European ancestry is relatively low (King *et al.*, 1998; Uitewaal *et al.*, 2004; Nolan *et al.*, 2011); in the USA, for populations of European, African, Hispanic and Pima Indian descent, the prevalence of T2D in both sexes is 7.6, 13, 17 and 50%, respectively (King *et al.*, 1998). It has been proposed that these differences in T2D susceptibility between European and non-European populations are the genetic and evolutionary consequences of geographical differences in the history of food consumption (Diamond, 2003; Corbett *et al.*, 2009).

The 'thrifty genotype theory' hypothesized that the T2D phenotype gives a survival advantage during periods of famine but is maladaptive in societies with high food abundance (Neel, 1962). Historical data show that, starting from about 1600, European societies became capable of efficiently avoiding famine, by redistributing any over-abundance of grain to areas of food scarcity (Rotberg *et al.*, 1985). Diamond suggested that as a result, Europeans should have undergone an epidemic in T2D starting several centuries before the present as a result of the new reliability of sufficient food supplies, and eliminated the most T2D-prone genotypes by processes of natural selection (Diamond, 2003).

Natural selection works through differences in reproductive success rather than simple differential survival. As fertility is a driving force behind evolution, infertility could be one of the underlying mechanisms through which T2D genotype is selected against, especially as T2D is a late-onset disease and therefore not directly acting on survival.

Therefore, we hypothesize that reduced fertility could be one of the underlying causes of the decreased T2D genotype frequencies in Europeans. However, it is unknown whether T2D is associated with reproductive problems earlier in life, although there is indirect evidence suggesting that patients with T2D experience reproductive problems before disease onset. Fertility problems are frequently followed by early menopause (Kok et al., 2003), and early menopause has been associated with premenopausal diagnosis of T2D (Dorman et al., 2001). Obesity, the most important risk factor for T2D, is associated with reduced fertility. Previously, a U-shaped association between BMI and relative risk of ovarian infertility was observed in the Nurses' Health Study II, with increased risk for ovarian infertility for women with a BMI <20 and $>24 \, \text{kg/m}^2$ (Rich-Edwards et al., 2002).

Thus far, it is unknown whether low fertility is indeed associated with the risk of developing T2D. Therefore, we assessed the association between measures of low fertility and T2D risk in the Prospect-European Prospective Investigation into Cancer and Nutrition (EPIC) cohort comprising 17 357 Dutch women.

Materials and Methods

Subjects

The Prospect-EPIC cohort is one of the two Dutch contributions to EPIC (Riboli, 1992). It is a prospective cohort study among 17 357 women aged 49–70 years who lived in Utrecht and vicinity and who participated in the breast cancer-screening programme between 1993 and 1997.

All participants gave their written informed consent and the study was approved by the Institutional Review Board. The design, sampling strategies and examination techniques of the cohort have been described previously (Boker et al., 2001).

Data collection

Baseline measurements
At baseline, all participants filled out detailed questionnaires on usual diet, reproductive history, presence of chronic diseases and related potential risk factors. Women underwent a brief medical examination and a blood sample was drawn.

Measures of reduced fertility
For each analysis, we used the following variables in an appropriate subpopulation: (i) subfertility, defined as a positive answer to the question whether a woman ever consulted a medical doctor for fertility problems, in all women who reported that they have tried to achieve pregnancy; (ii) nulliparity, in women who reported that they have tried to achieve pregnancy; (iii) having only one child, in all parous women; (iv) at least one miscarriage, in all women who were ever pregnant; (v) time interval > 5 years between birth of first and second child, in women with at least two live born children and (vi) menstrual cycle irregularity, in all women reporting on menstrual cycle pattern. The information on menstrual cycle pattern concerned the period between age 30 and 40 years in which women were not using oral contraceptives. Irregularity of the menstrual cycle pattern was self-defined.

Potential covariates
Age, BMI, waist circumference, physical activity, socio-economical status, smoking and alcohol use could potentially influence both fertility and susceptibility to T2D. Therefore, we adjusted our analyses for age, alcohol intake, physical activity, smoking and socio-economic status. We additionally adjusted for oral contraceptive use as this obviously affects reproductive outcome and oral contraceptive use might affect sex steroid hormone levels in women using them, which could influence T2D risk. To assess whether BMI and waist circumference influence the association between fertility and T2D risk, we adjusted for these measures in subsequent models.

Body weight was measured to the nearest 0.5 kg, while wearing light indoor clothing without shoes using a floor scale (Seca, Atlanta, GA, USA). Additionally, height, waist circumference and hip circumference were measured. BMI was expressed as kg/m^2.

Alcohol consumption was assessed by a validated food frequency questionnaire. Baseline alcohol intake was determined by multiplying the consumption of each beverage by its ethanol content and was expressed as g/week. Subsequently, we categorized subjects into four categories of alcohol consumption: <0.05, $0.05–5.5$, $5.5–10.5$ and >10.5 g/week.

Duration and types of physical activity during the year preceding study recruitment were assessed by a set of questions that was used in all EPIC cohorts. By combining occupational physical activity with time spent on cycling and sporting in summer and winter, the validated Cambridge Physical Activity Index (Wareham et al., 2003) was calculated (Beulens et al., 2010a,b). Based on this index, participants were assigned to one of four categories: inactive, moderately inactive, moderately active and active.

Smoking behaviour was categorized as no, former or current smokers.

To define socio-economic status, the highest attained level of education of the participants was used and classified into three categories: low (primary education up to completing intermediate vocational education), middle (up to higher secondary education) and high (those with higher vocational education and university).

The number of years of oral contraception use was self-reported, and participants were assigned to one of four groups: never, 1–4, 4–10 and >10 years.

Missing value analyses
Missing values for BMI, waist circumference, alcohol intake, smoking, gestational diabetes, socio-economic status, years of oral contraceptives use, number of miscarriages and age of menarche were imputed using multiple imputation (van der Heijden et al., 2006) and repeated five times to account for uncertainties in imputed data. None of the variables had $>5\%$ missing values; the percentage of missing values ranged from 0.1% for BMI to 2.9% for years of oral contraceptives use.

Morbidity and mortality follow-up
Occurrence of prevalent T2D was obtained via self-report in the baseline questionnaire and through linkage to the Dutch register of hospital discharge diagnoses (HDD) for the period before enrolment. Information on incident T2D was obtained via self-report in two follow-up questionnaires sent to the participants within intervals of 3–5 years, linkage to the Dutch register of HDD and a mailed urinary glucose strip test (part of the cohort) (Sluijs et al., 2010). Potential prevalent and incident cases of T2D were verified against information from the participants' general practitioner or pharmacist through mailed questionnaires. T2D was defined as being present when the general practitioner or pharmacist confirmed the diagnosis. Information on vital status was obtained through linkage with the municipal administration registries (Herings et al., 1992). Causes of death were obtained from the Dutch Central Bureau of Statistics, coded according to the International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10). For our analyses, T2D was the end-point of interest and follow up ended at

the date of diagnosis or at the date of death. All others were censored on 1 January 2006.

Data analysis

Population characteristics were described using mean and SD (for normally distributed variables) and number and frequency (for categorical variables).

The person-time for each woman was calculated from birth to the month of diagnosis of the end-point (T2D), the month of death from other causes or the end of follow-up (1 January 2006). In prospective cohort studies, the person-time is usually calculated from baseline, therefore only including incident T2D cases. As our variables for measures of low fertility were established long before T2D onset in cases, we chose to calculate person-time from birth. This allowed us to include an extra 332 prevalent T2D cases for analyses, which would otherwise be excluded. Person-time from birth has been used in another study where a variable (age of menarche) was estimated before baseline (Lakshman *et al.*, 2008). We additionally studied the association of low fertility and T2D risk with person-time calculated from baseline, to explore the effect of using a person-time from baseline versus from birth. Two approaches were used to assess the validity of the proportional hazards assumption. First, the assumption was assessed by log-minus-log-survival function and found to hold. Second, to confirm the assumption of proportionality, time-dependent covariate analysis was used. The time-dependent covariates were not statistically significant for all but one covariate, suggesting that the proportional hazards assumption is reasonable. The time-dependent alcohol use covariate was statistically significant ($P = 0.009$), however, removing alcohol use from the model did not change our results.

Hazard ratios (HRs) and 95% confidence intervals (CIs) for risk on T2D were estimated using Cox regression analysis. We used a stepwise approach to adjust for potential confounders and study the role of the potential intermediate factors, BMI and waist circumference, using five multivariate models: model 1, including age at baseline (for analysis using person-time calculated from baseline); model 2, including potential confounders age, alcohol intake, physical activity, smoking, socio-economic status and oral contraceptives use; model 3, including all confounders from model 2 and BMI; model 4, including all confounders from model 2 and waist circumference.

All statistical analyses were performed using the Statistical Package for the Social Sciences (version 19). Statistical significance was set at $P < 0.05$.

Results

Table I shows the baseline characteristics of the population included in this study. The study had a mean follow-up of 9.1 ± 3.6 years and comprised 157 964 person-years. Calculating follow-up time from birth resulted in a mean follow-up of 66.8 ± 6.7 years with a corresponding 1 160 428 person-years. The mean age of the study group at baseline was 57.1 ± 6.0 years. In total, the study contained 867 verified T2D patients; 332 prevalent and 535 incident cases.

Table II shows the relation between different measures of low fertility and T2D risk. Unadjusted and adjusted HRs were all small, with 95% CIs often including 1.00 or barely missing 1.00. When fully adjusted, there was no association between any of the measures of low fertility and T2D risk.

Out of the 15 707 women who wanted to get pregnant, 1940 (12.4%) consulted a physician for fertility problems and 133 women (0.8%) did not report whether or not they had a consultation. Consulting a physician for fertility problems was not associated with risk

Table I Baseline characteristics of 17 357 Dutch women from the Prospect-EPIC cohort.

	Mean ± SD
Follow-up time from birth (year)	66.9 ± 6.7
Age at intake (year)	57.1 ± 6.0
BMI (kg/m^2)	26.0 ± 4.10
Waist circumference (cm)	83.8 ± 10.2
Alcohol intake (g/week)	9.1 ± 12.6
Oral contraceptive pill use (year)	5.4 ± 6.8
Live born children	2.4 ± 1.5
Smoking, *n* (%)	
Current smoker	3790 (21.8)
Former smoker	6073 (35.0)
Non-smoker	7494 (43.2)
Physical activity, *n* (%)	
Inactive	1301 (7.5)
Moderate inactive	4612 (26.6)
Moderate active	4437 (25.6)
Active	7007 (40.4)
Educational level, *n* (%)	
Low	13311 (76.7)
Medium	1249 (7.2)
High	2270 (13.1)

of T2D. Of these 15 707 women, 700 (4.5%) remained childless. No relation was found between nulliparity and T2D risk. Furthermore, 3946 (25.1%) women in this group had one or more miscarriage(s), with an average of 1.4 (± 0.9) miscarriages and a maximum of 10 miscarriages. Women who had one or more miscarriage showed no different risk for T2D compared with women who did not have a miscarriage.

Of the 15 007 women who did have children, 1470 (9.8%) women were uniparous. Compared with women with two or more children, women with only one child had a decreased risk for T2D, with a multivariate adjusted HR of 0.78 (95% CI 0.60–1.02), although not significant. The average time interval between the first and second child was 32.7 (± 20.4) months. Women with a time interval of 5 years or more between their first and second child had an increased risk for T2D, with an unadjusted HR of 1.27 (95% CI 1.00–1.62) compared with women with two or more children. However, after multivariate adjustment, this association was no longer significant, with a multivariate adjusted HR of 1.11 (95% CI 0.87–1.41).

A total of 17 357 women reported on regularity of natural menstruation between the age of 30 and 40 years, of whom 1947 (11.4%) reported having an irregular menstrual cycle. Compared with women with regular cycle length, women with irregular menstrual cycles had an increased risk for T2D, with an unadjusted HR of 1.27 (95% CI 1.04–1.54). However, after adjustment for multiple covariates, the association between irregular menstrual cycles and increased T2D risk was no longer significant.

The results for the analyses using only incident cases were very similar to the results including both incident and prevalent cases

(Table III). The effect of exposures and of confounders on the outcome in Cox regression will be estimated on the risk set at the first and subsequent events. The reproductive events and the T2D

Table II Hazard ratios (HR) for T2D by various measures of low fertility in Prospect-EPIC women with age as follow-up time.

	HR	HR (95% CI)
Ever a consult for sub- or infertility	No	Yes
Subjects (n)	13 634	1940
T2D cases (%)	701 (5.1%)	83 (4.3%)
Model 1[a]: unadjusted	1	0.88 (0.70–1.11)
Model 2[b]: multiple confounders	1	0.97 (0.77–1.22)
Model 3[c]: model 2 + BMI	1	1.06 (0.85–1.34)
Model 4[d]: model 2 + WC	1	1.05 (0.84–1.32)
Nulliparity	No	Yes
Subjects (n)	15 007	700
T2D cases (%)	762 (5.1%)	31 (4.4%)
Model 1[a]: unadjusted	1	0.88 (0.62–1.26)
Model 2[b]: multiple confounders	1	0.99 (0.69–1.43)
Model 3[c]: model 2 + BMI	1	1.04 (0.72–1.49)
Model 4[d]: model 2 + WC	1	1.06 (0.74–1.52)
Uniparity	No	Yes
Subjects (n)	13 537	1470
T2D cases (%)	702 (5.2%)	60 (4.1%)
Model 1[a]: unadjusted	1	0.82 (0.63–1.07)
Model 2[b]: multiple confounders	1	0.78 (0.60–1.02)
Model 3[c]: model 2 + BMI	1	0.84 (0.64–1.09)
Model 4[d]: model 2 + WC	1	0.82 (0.63–1.08)
Ever a miscarriage	No	Yes
Subjects (n)	11 761	3946
T2D cases (%)	569 (4.8%)	224 (5.7%)
Model 1[a]: unadjusted	1	1.11 (0.96–1.30)
Model 2[b]: multiple confounders	1	1.11 (0.95–1.29)
Model 3[c]: model 2 + BMI	1	1.05 (0.90–1.22)
Model 4[d]: model 2 + WC	1	1.00 (0.86–1.17)
Interval first and second child > 5 years	No	Yes
Subjects (n)	12 322	1078
T2D cases (%)	623 (5.1%)	73 (6.8%)
Model 1[a]: unadjusted	1	1.27 (1.00–1.62)
Model 2[b]: multiple confounders	1	1.11 (0.87–1.41)
Model 3[c]: model 2 + BMI	1	1.08 (0.84–1.37)
Model 4[d]: model 2 + WC	1	1.05 (0.82–1.34)
Irregular menstrual cycle	No	Yes
Subjects (n)	15 410	1947
T2D cases (%)	750 (4.9%)	117 (6%)
Model 1[a]: unadjusted	1	1.27 (1.04–1.54)
Model 2[b]: multiple confounders	1	1.06 (0.83–1.36)
Model 3[c]: model 2 + BMI	1	1.09 (0.86–1.40)

Continued

Table II *Continued*

	HR	HR (95% CI)
Model 4[d]: model 2 + WC	1	1.08 (0.84–1.38)

Women with a time interval of 5 years or more between their first and second child had an increased risk for T2D compared with women with two or more children. However, after multivariate adjustment, this association was no longer significant. Compared with women with regular cycle length, women with irregular menstrual cycles had an increased risk for T2D. However, after adjustment for multiple covariates, the association between irregular menstrual cycles and increased T2D risk was no longer significant.
CI, confidence interval.
[a]Model 1 = Unadjusted.
[b]Model 2 = Adjusted for smoking (never, past and current), alcohol intake (<0.05, 0.05–5.5, 5.5–10.5 and >10.5 g/w), socio-economic status (low, middle and high), oral contraceptive pill years (never, 1–4, 4–10 and >10 years) and physical activity (inactive, moderate inactive, moderate active and active).
[c]Model 3 = Model 2 plus BMI (continuous).
[d]Model 4 = Model 2 plus waist circumference (WC; continuous).

outcome did not overlap in most cases, hence not changing the risk set. Therefore, we decided to present the results using both incident and prevalent cases.

Discussion

In this large cohort of 17 357 women, measures of low fertility were not associated with the risk of developing T2D. Therefore, we could find no evidence to support our hypothesis that reduced fertility is one of the underlying causes of the decreased T2D genotype frequencies in Europeans. To our knowledge, this is the first study to investigate the association between various measures of low fertility and future T2D risk in a prospective cohort. Our study was powered to show an association of clinical relevance between measures of low fertility and T2D, as we had 80% power to detect a HR of 1.4 for determinant variables with a 10% prevalence, when including both prevalent and incidence cases.

Before discussing the data, some strengths and limitations need to be highlighted. The main advantages of this study are its prospective nature, the long follow-up time and the large sample size. Furthermore, the women were extensively questioned on their reproductive history and the participants' general practitioner or pharmacist validated cases of T2D.

Prospect-EPIC is a prospective cohort study among women who participated in the breast cancer-screening programme between 1993 and 1997. In the Netherlands, all women aged 50–69 years are offered a free biennial breast cancer screen examination and the participation rate is high. Between 1990 and 1995, the overall attendance rate of females in the Netherlands for breast cancer screening was 77.5% (Fracheboud *et al.*, 1998). Establishing whether the Prospect-EPIC cohort is representative of the general European female population is difficult. It is known that volunteers for epidemiology studies are, in general, better educated and use health services less often compared with non-volunteers. However, using a restricted source population for a cohort study will produce only relatively weak bias in estimates of the exposure-disease associations (Pizzi *et al.*, 2011). Also, many baseline characteristics in Prospect-EPIC are similar to other European cohorts (Boker *et al.*, 2001). We therefore

Table III Hazard ratios for T2D by various measures of low fertility in Prospect-EPIC women with person-time from baseline.

	HR	HR (95% CI)
Ever a consult for sub- or infertility	No	Yes
Subjects (n)	13 365	1908
T2D cases (%)	432 (3.2)	51 (2.7)
Model 1[a]: age	1	0.88 (0.67–1.16)
Model 2[b]: multiple confounders	1	0.95 (0.71–1.25)
Model 3[c]: model 2 + BMI	1	1.04 (0.79–1.38)
Model 4[d]: model 2 + WC	1	1.01 (0.77–1.34)
Nulliparity	No	Yes
Subjects (n)	14 717	683
T2D cases (%)	472 (3.2%)	14 (2%)
Model 1[a]: age	1	0.72 (0.44–1.17)
Model 2[b]: multiple confounders	1	0.80 (0.49–1.30)
Model 3[c]: model 2 + BMI	1	0.86 (0.53–1.39)
Model 4[d]: model 2 + WC	1	0.87 (0.53–1.41)
Uniparity	No	Yes
Subjects (n)	13 265	1452
T2D cases (%)	430 (3.2%)	42 (2.9%)
Model 1[a]: age	1	0.90 (0.66–1.23)
Model 2[b]: multiple confounders	1	0.84 (0.61–1.15)
Model 3[c]: model 2 + BMI	1	0.90 (0.66–1.24)
Model 4[d]: model 2 + WC	1	0.89 (0.65–1.22)
Ever a miscarriage	No	Yes
Subjects (n)	11 537	3863
T2D cases (%)	345 (3.0%)	141 (3.7%)
Model 1[a]: age	1	1.17 (0.97–1.42)
Model 2[b]: multiple confounders	1	1.18 (0.98–1.43)
Model 3[c]: model 2 + BMI	1	1.13 (0.94–1.37)
Model 4[d]: model 2 + WC	1	1.09 (0.90–1.32)
Interval first and second child > 5 years	No	Yes
Subjects (n)	12 083	1045
T2D cases (%)	385 (3.2%)	40 (3.8%)
Model 1[a]: age	1	1.19 (0.88–1.62)
Model 2[b]: multiple confounders	1	1.05 (0.77–1.44)
Model 3[c]: model 2 + BMI	1	1.02 (0.75–1.39)
Model 4[d]: model 2 + WC	1	0.99 (0.72–1.34)
Irregular menstrual cycle	No	Yes
Subjects (n)	15 125	1900
T2D cases (%)	465 (3.1%)	70 (3.7%)
Model 1[a]: age	1	1.18 (0.92–1.51)
Model 2[b]: multiple confounders	1	1.09 (0.85–1.40)
Model 3[c]: model 2 + BMI	1	1.14 (0.89–1.46)
Model 4[d]: model 2 + WC	1	1.11 (0.86–1.42)

The results for the analyses using only incident cases (Table III) were very similar to the results including both incident and prevalent cases (Table II).

[a]Model 1 = Adjusted for age at baseline (continuous).
[b]Model 2 = Adjusted for age at baseline (continuous), smoking (never, past and current), alcohol intake (<0.05, 0.05–5.5, 5.5–10.5 and >10.5 g/w), socio-economic status (low, middle and high), oral contraceptive pill years (never, 1–4 , 4–10 and >10 years) and physical activity (inactive, moderate inactive, moderate active and active).
[c]Model 3 = Model 2 plus BMI (continuous).
[d]Model 4 = Model 2 plus WC (WC; continuous).

feel that Prospect-EPIC is a representative cohort for European women.

The variables we used in this study as measures of low fertility have been used in previous studies as reproductive characters that reflect low fertility (Cramer *et al.*, 1995; Hardy and Kuh, 1999; Kok *et al.*, 2003). Associations between early age at menopause and a higher frequency of all characteristics that we use in this study, reflecting low fertility, were reported previously (Kok *et al.*, 2003).

Although the mean age of participants at baseline was substantially older than age during the investigated reproduction time, most measures of low fertility are reflecting the current family situation of women. As this is very stable over time, it will make recall errors very unlikely, when women are asked to answer questions on this topic. A previous study in women on the accuracy of recall of spontaneous abortion showed that 75% of recorded abortions were recalled (Wilcox and Horney, 1984). Gestational age at time of abortion was the major determinant of recall, with early abortions remembered less often, while time since abortion affected the recall to a lesser extent. Recall of menstrual cycle length is probably the least reliable of all measures of low fertility used in this study (Bean *et al.*, 1979).

The variables 'having consulted a physician for fertility problems', 'nulliparity', 'uniparity' and 'a long time interval between the birth of the first and the second child' could also have been caused by male infertility. However, the associations between measures of low fertility and T2D risk did not change when we excluded women with sub- or infertile partners (data available on request). Although some misclassification of the fertility status of the women cannot be excluded, this is unlikely to have an effect on the results, since misclassification of low fertile or infertile women occurred independently of T2D.

The variable 'time interval between birth of first and second child' is a substitute for time to pregnancy, which is widely used to estimate the degree of low fertility (Greenhall and Vessey, 1990). In this study, we were unable to directly determine time to pregnancy in our cohort, and instead used the interval between first and second child as we expect that this time is, for the most part, unintentional waiting time. However, this subfertility is most likely of relatively minor magnitude because all of these women were able to conceive at least twice. It should also be mentioned that while the interpregnancy interval may be a marker of low fertility, it could also be a marker of high fertility in a subgroup, as it could represent accidental pregnancies in women who were satisfied with having one child.

In previous studies long or highly irregular menstrual cycles have been associated with insulin resistance, higher glucose levels and increased risk of T2D (Weiss *et al.*, 1994; Roumain *et al.*, 1998; Gast *et al.*, 2010). We previously reported that, compared with women with a regular cycle length of 27–29 days, women with irregular menstrual cycles tended to be (non-significant) at increased risk for T2D, and had a significantly increased risk of coronary heart disease (Gast *et al.*, 2010). In the present study, we showed that the association with T2D slightly strengthened after adjusting for both BMI and waist circumference, separately. However, the nature of any link between irregular menstrual cycles and T2D remains unknown. In women with irregular menstrual cycles neither the association with T2D nor the association with coronary heart disease could be explained by metabolic risk factors or altered hormone levels (Gast *et al.*, 2010).

In the Prospect cohort, 91.2% of the patients with T2D for whom age of menopause was known were diagnosed after menopause. As the developing epidemic of obesity at a younger age will result in a substantial reduction in the age of onset of T2D, which is already emerging in women of childbearing age, it is important to further investigate the association between the reduced fertility and premenopausal T2D. Previous studies provide some evidence for the connection between infertility and premenopausal T2D. First of all, one common cause of low fertility, namely polycystic ovary syndrome (PCOS), is already known to be associated with impaired glucose tolerance and T2D in adolescent girls and premenopausal women (Legro et al., 1999; Moran et al., 2010). PCOS is a heritable form of ovarian infertility that clinically affects 5–10% of women of reproductive age and is characterized by a long history of chronic anovulation in association with insulin resistance and androgen excess (Azziz et al., 2005, Rotterdam ESHRE/ASRM, 2004). Second, reproductive abnormalities are often part of the metabolic syndrome when it occurs in premenopausal women (Sam and Dunaif, 2003). Metabolic syndrome is recognized as a major risk factor for T2D (Reusch, 2002). Third, pregnancy losses, predominantly through stillbirth, are high in women with type 1 diabetes and T2D (Macintosh et al., 2006). However, it is unknown whether T2D-associated phenotypes cause low fertility or whether low fertility are markers for unknown factors increasing T2D risk in menopausal women. As our data show that low fertility does not predict subsequent development of T2D, it is tempting to speculate that premenopausal T2D is causal for low fertility, rather than the other way around. Unfortunately, in this Prospect-EPIC study we were not able to study the association between reduced fertility and premenopausal T2D risk, owing to the low number of premenopausal T2D cases.

Our data show that general measures of low fertility are not associated with T2D later in life. As previous studies provide some evidence for a connection between low fertility and premenopausal T2D, it is tempting to speculate that premenopausal T2D is causal for reduced fertility rather than the other way around. Therefore, the association between low fertility and risk of premenopausal T2D should be further studied, as alongside the developing epidemic of obesity there is a substantial reduction in the age of onset of T2D and its emergence in women of childbearing age.

Authors' roles

C.C.E., N.C.O. and Y.T.vd S. designed the study. C.C.E. and M.J.C.E. performed statistical analysis. D.E.G. provided data. C.C.E., N.C.O., C.W. and Y.T.vd S. wrote the manuscript.

Funding

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