

Turno Nocturno Rotativo y Edad Menopáusica

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Resumen e Introducción

Resumen

Pregunta del estudio: ¿Cómo se relacionan los horarios de turnos nocturnos rotativos con la edad en la menopausia entre una gran cohorte nacional de enfermeras que trabajan por turnos?

Respuesta resumida: nuestros hallazgos sugieren que trabajar turnos nocturnos rotativos con suficiente frecuencia puede acelerar modestamente la senescencia reproductiva entre las mujeres que ya pueden estar predispuestas a una menopausia más temprana.

Lo que ya se sabe: una edad más joven en la menopausia se ha asociado con un mayor riesgo de resultados adversos para la salud, en particular los relacionados con la reproducción. El trabajo nocturno se ha asociado con disfunción reproductiva, incluida la interrupción de los patrones del ciclo menstrual.

Diseño del estudio, tamaño, duración: este estudio de cohorte se realizó entre 80 840 mujeres del Estudio de salud de enfermeras 2 (NHS2), con un seguimiento prospectivo desde 1991 hasta 2013. La pérdida del seguimiento del NHS2 se estima en ser <10%.

Participantes / materiales, entorno, métodos: Se evaluó la asociación entre el trabajo nocturno rotativo acumulado y el turno de la noche actual en la menopausia natural durante los 22 años de seguimiento (1991–2013). Se utilizaron modelos de riesgos proporcionales de Cox para estimar los índices de riesgo (HR) para la menopausia, ajustados por edad, estado de fumador, índice de masa corporal, actividad física, consumo de alcohol, factores reproductivos y uso de hormonas exógenas.

Principales resultados y el papel del azar: durante el seguimiento, 27 456 mujeres (34%) alcanzaron la menopausia natural. Las mujeres que trabajaron 20 o más meses de turnos nocturnos rotativos en los 2 años anteriores tuvieron un mayor riesgo de menopausia temprana (multivariable ajustado (MV) -HR = 1.09, IC 95%: 1.02–1.16) en comparación con las mujeres sin turno de noche trabajo por turnos. Este riesgo fue mayor entre las mujeres sometidas a menopausia o censuradas de otro modo menores de 45 años (MV-HR = 1.25, IC 95%: 1.08–1.46), que para aquellas que continuaron en el estudio cuando > 45 años de edad (MV-HR = 1.05, IC del 95%: 0.99–1.13). Trabajar 10 o más años de trabajo nocturno rotativo acumulado también se asoció con un mayor riesgo de menopausia entre las mujeres que alcanzan la menopausia menores de 45 años (MV-HR_{10–19 años} = 1.22, IC 95%: 1.03–1.44; MV-HR_{≥20 años} = 1.73, IC 95%: 0.90–3.35), aunque no es mayor de 45 años (MV-HR_{10–19 años} = 1.04, IC 95%: 0.99–1.10; MV-HR_{≥20 años} = 1.01, 95% CI: 0.89–1.15).

Limitaciones, razones para la precaución: El grado en que los efectos observados de los turnos nocturnos rotatorios en la edad de la menopausia natural se deben a la interrupción circadiana, en lugar de a la fatiga y al estrés asociados con los horarios más exigentes de trabajo, es incierto debido a la posibilidad de confusión residual por estos factores.

Wider Implications of the Findings: This is the first study to assess the effects of night work on menopausal timing among a larger national cohort of shift working women. Women already prone to earlier menopause may further truncate their reproductive lifetime by working schedules comprising day as well as night shifts.

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Introduction

Age at natural menopause has important implications on women's health. Earlier menopause has been associated with increased risk of cardiovascular disease, osteoporosis, shorter life expectancy and even cognitive decline while women who go through menopause when they are older are at greater risk for breast, endometrial and ovarian cancers (Gold, 2011; Ruth *et al.*, 2016). Considerable variation exists in age at natural menopause, the reasons for which are not fully understood. Menopause occurs when a minimum number of oocytes cease to be viable, the variation in timing of which is thought to be chiefly dependent on rates of follicle atresia (Gougeon, 1996; Gold, 2011). While a strong genetic component is undeniable (He *et al.*, 2009; Stolk *et al.*, 2012, 2009), familial studies estimating variation in age at menopause attributable to heritable factors (Snieder *et al.*, 1998; de Bruin *et al.*, 2001; van Asselt *et al.*, 2004; Murabito *et al.*, 2005)

have been highly variable and suggest environmental exposures contribute substantially to age at menopause. Though there is debate in the epidemiologic literature, modifiable risk factors for age at menopause have been identified. The strongest of these include smoking-related exposures, lower parity and socioeconomic status, while evidence for other candidates such as diet, body fat composition or oral contraceptive use have been less conclusive (Gold, 2011; Ruth *et al.*, 2016). To date, these factors are unlikely to explain a substantial portion of variation attributable to exogenous causes (Voorhuis *et al.*, 2010).

A factor that may impact age at menopause is night work, previously linked to adverse reproductive outcomes including changes in menstrual cycle patterns (Chung *et al.*, 2005; Su *et al.*, 2008; Lawson *et al.*, 2011) as well as higher risk of preterm delivery (Bonzini *et al.*, 2007) and miscarriage (Fernandez *et al.*, 2016), suggesting an effect of circadian disruption on ovulation and fertility. The increased risk of hormonally triggered cancers among long-term night shift workers, including endometrial (Viswanathan *et al.*, 2007) and breast cancer (Schernhammer *et al.*, 2001, 2006), lends further support to the notion that circadian perturbations may alter reproductive signaling.

Desynchronization from environmental circadian cues, as experienced by night workers, may impact menopausal timing through chronic dysregulation of gonadal function. Exposure to artificial light during dark hours suppresses and alters the timing of nocturnal pineal melatonin secretion (Brainard *et al.*, 1997). Though causal relationships have not been substantiated, observations that endogenous melatonin decreases during puberty (Waldhauser *et al.*, 1984; Attanasio *et al.*, 1985), that higher levels are coincident with functional hypogonadism (Berga *et al.*, 1988; Laughlin *et al.*, 1991), or that circulating melatonin has been inversely correlated with estrogen levels in amenorrhoeic women (Okatani and Sagara, 1994) could suggest an overall inhibitory effect of melatonin on ovarian activity. Thus, a decrease in nocturnal melatonin may promote gonadal activity, potentially delaying menopause. Conversely, indications that melatonin protects follicle integrity (Cruz *et al.*, 2014; Tamura *et al.*, 2017), thereby imparting resistance to atresia, and evidence of the importance of synchronized endocrine signaling and clock gene expression across central and peripheral targets on mammalian ovarian function (Sellix, 2015; Simonneaux and Bahougne, 2015), suggest night work-associated circadian disruption may translate into earlier menopause. However, whether night shift work affects the age at onset of menopause has not been studied to date.

We investigated the association between rotating night shift work and age at natural menopause within the Nurses' Health Study 2 (NHS2) cohort. Our findings will add insight on the impact of night work schedules on ovarian function and further elucidate the role of circadian regulation in human reproduction.

Materials and Methods

Study Population

The NHS2 is an ongoing prospective cohort of 116 429 female registered nurses in the USA between ages 25 and 42 at its start in 1989 (Willett, 2013). Biennial questionnaires have been collected since baseline, capturing lifestyle, environmental and occupational exposures, as well as medication use and medical conditions; follow-up to date exceeds 90%. The Institutional Review Board of Brigham and Women's Hospital (Boston, MA) approved this study, and informed consent was implied by the return of the initial questionnaire.

Rotating Night Shift Work Exposure

Rotating night shift work, to be distinguished from exclusive night work, signifies schedules that include working nights concurrently with either evenings or days. Hypothesized to be more disruptive to circadian-regulated processes than exclusive night work, it has been studied previously as a risk factor for adverse reproduction-linked outcomes in Nurses' Health Study cohorts (Schernhammer *et al.*, 2001, 2006). The 1989 NHS2 questionnaire queried number of prior years (1–2, 3–5, 6–9, 10–14, 15–19 or 20+) worked with the following item: 'What is the total number of years during which you worked rotating night shifts (at least 3 nights/month in addition to days/evenings in that month)?'. Subsequent 1991, 1993, 1995, 1997, 2001, 2005, 2007 and 2011 questionnaires queried number of months (none, 1–4, 5–9, 10–14, 15–19 and 20 or more) in which at least 3 nights per month, in addition to days and evenings, had been worked in the past 2 years. The 2001 and 2005 questionnaires queried multiple consecutive 2-year intervals so that complete rotating night shift work exposure through 2005 was captured. As rotating shift work exposure was not captured in 2009, values were carried forward from the previous questionnaire for those who reported working current jobs involving rotating night shifts. Women who did not report working such jobs were assigned zero months of rotating night shifts for 2007 through 2009.

Cumulative months worked rotating night shifts prior to 1989 was estimated by taking the midpoint of each category and multiplying by 12. Women who reported working 20 or more years were assigned 240 months of cumulative rotating night shift work. Cumulative months worked over follow-up was estimated by adding the midpoints of each category across biennial questionnaires; for the open-ended '20 or more' category, we conservatively assigned 20 months. In the event of missing data, rotating night shift work was carried forward for one questionnaire cycle.

Age at Natural Menopause

On each biennial NHS2 questionnaire, participants were asked whether their menstrual periods had ceased, at what age, and if this was due to natural menopause, chemotherapy or radiation, or surgery. When reported age at menopause was inconsistent across questionnaires, the first reported age was used.

Ascertainment of Covariates

Potential confounders were selected a priori. Age, smoking (Mikkelsen *et al.*, 2007; Pokoradi *et al.*, 2011; Sarac *et al.*, 2011), parity (Parazzini, 2007), body mass index (Kato *et al.*, 1998), oral contraceptive use (Pokoradi *et al.*, 2011), age at menarche (Parazzini, 2007), alcohol consumption (Torgerson *et al.*, 1997; Brett and Cooper, 2003) and physical activity (Nagata *et al.*, 2012) have previously been associated, to varying degrees, with timing of natural menopause. Breastfeeding has also been speculated to impact this process (Weinstein *et al.*, 2003). Sleep duration was also considered as a potential confounder.

Statistical Analyses

Women were excluded if they did not return the study baseline 1991 questionnaire or had missing or incomplete records for rotating night shift work exposure at, or prior to study baseline. Further exclusion criteria included natural or medically induced menopause, use of menopausal hormone therapy (HT), or cancer diagnosis (excluding non-melanoma skin cancer) on, or prior to the 1993 questionnaire.

Cox proportional hazards models were used to estimate the effect of rotating night shift work on timing of natural menopause. The time metameter was age in months, effectively controlling all models for age (i.e. all models were age-adjusted). Women entered the cohort at their age at the 1993 biennial questionnaire return date. A counting process data structure (Therneau, 2000) was used to handle time-dependent covariates and accommodate delayed entry, permitting women to enter the cohort at differing ages. The main exposure, rotating night shift work and covariate values, were updated at each biennial questionnaire.

In our main exposure, we defined rotating night shift work as recent number of months worked rotating night shifts, i.e. during the 2-year interval prior to the preceding questionnaire return to ensure temporality (i.e. shift work occurred prior to reported age at menopause). Secondly, we updated 'lifelong' years of rotating night shifts worked to the end of the prior 2-year questionnaire cycle. Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Women were censored at cancer diagnosis, death, hysterectomy, bilateral oophorectomy, report of periods ceasing due to chemotherapy or radiation, or initiation of HT use. Censoring at cancer diagnosis and HT use was due to potential effects of cancer or chemotherapy (Sklar, 2005) on menstruation, and exogenous hormone medications interfering with menopausal timing (Jernstrom *et al.*, 2003) and inducing menstruation (Furness *et al.*, 2009), respectively. Further, women were censored for failing to complete two consecutive questionnaires, and if missing rotating night shift data on two consecutive questionnaires.

Sensitivity Analyses

Violations of the proportional hazards assumption were assessed visually (Hosmer, 1999). The relative hazard of menopause for both recent and lifelong rotating shift work exposure definitions varied with age (i.e. the risk of earlier menopause among rotating night shift workers relative to women who did not work these schedules was not constant as women grew older); we, therefore, divided the time metameter (i.e. age) at the average age in this cohort, i.e. 45 years, and present stratified results. Age- and multivariable-adjusted hazard ratios (HRs) were estimated for women younger and older than 45 years for both exposure definitions. We plotted two sets of multivariable-adjusted cumulative probability functions of remaining premenopausal comparing only women who did not work any months of rotating night shifts in the prior two years to those who worked 20 or more. The first set included women 30–45 years old, the second, those 45 years and older. Due to literature suggesting that diabetes may increase risk of earlier menopause, sensitivity analyses were conducted excluding women with Type I and Type II diabetes prior to baseline (Wellons *et al.*, 2017). As smoking is one of the most reliable exogenous determinants of menopausal timing, we investigated the impact of substituting smoking status with pack-years.

Results

As of the 1991 questionnaire return, 80 840 women met eligibility criteria. Of these, 61% of women reported working rotating night shifts prior to 1989 and 38% reported working rotating night shifts between 1989 and 2011. Of women who reported working rotating night shifts prior to 1989, the mean (SD) cumulative duration was 70.5 (67.5) months. Of those who reported rotating night shifts only after the 1989 baseline, the mean cumulative duration worked over follow-up was 36.9 (46.6) months. Working rotating night schedules was less common with age. While 23% of women under 40 years reported working any rotating night shifts in the previous 2 years, only 8% over 50 years did so.

The distribution of covariates by duration of rotating night shift work between 1989 and 1991 questionnaire return dates is shown in . Women who worked any rotating night shifts during this interval were more likely to be current smokers. Rotating night shift workers were also younger (mean difference of 1.3 years), more physically active (median increase of 0.8 metabolic equivalents/week) and experienced less sleep (i.e. <7 h/night). Finally, they were more often nulliparous, though when they had children, did so at a younger age.

Table I. Age and age-standardized characteristics in 1991 according to months of rotating night shifts worked among 80 840 women in the Nurses' Health Study 2.

Characteristic	Rotating night shift work between 1989 and 1991			
	None	1–9 months	10–19 months	≥20 months
N	62 205	8768	4077	5790

Age, years (mean, SD)	36.5 (4.5)	35.2 (4.7)	34.9 (4.7)	35.5 (4.7)
Alcohol consumption, g/d (median, quartiles)	0.9 (0–3.5)	0.9 (0–3.5)	0.9 (0–3.5)	0.9 (0–2.9)
BMI, kg/m ² (mean, SD)	24.2 (5.0)	25.2 (5.6)	25.4 (5.7)	25.6 (5.9)
Physical Activity, METs [‡] /wk (median, IQR)	12.5 (5.0–26.5)	12.7 (5.0–27.3)	14.1 (5.7–30.2)	13.8 (5.4–30.2)
Smoking Status (%)				
never smoker	67	64	64	64
past smoker	22	21	23	20
current smoker	11	15	14	15
Oral contraceptive use (%)				
Never used	17	16	15	17
1–47 mos	41	42	43	42
48–95 mos	25	24	25	24
≥96 mos	17	17	17	17
Parity (%)				
Nulliparous	26	32	28	28
1 child	19	17	17	16
2 children	35	31	32	34
3+ children	20	20	23	22
Age at first birth (%) [†]				
Nulliparous	26	32	28	28
≤20 y	6	9	10	10
21–25 y	28	28	29	30
26–30 y	31	24	26	26
>30 y	10	7	7	6
Total time breast fed (%) [†]				
Never breast fed	25	27	28	30
Breast fed ≤ 1 y	34	35	32	32
Breast fed > 1 y	41	37	40	38
Sleep, h/night (%)				
<7	27	34	34	39
7–8	68	62	62	56
≥9	5	4	5	5
Age at menarche (%)				
<12 y	24	25	25	25
12–13 y	59	58	57	56
≥14 y	18	18	18	19

Values are means (SD), median (quartiles) or percentages of non-missing values. Category percentages may not sum to 100 due to rounding.

[†]Parous women only.

[‡]Metabolic equivalent tasks.

Of the 80 840 eligible women at baseline 49 748 were right-censored prior to end of follow-up, 33 450 of which was due to rotating shift work exposure data missing for two consecutive cycles ($n = 19\,742$) and HT use ($n = 13\,708$). Of those not censored, 27 456 (57%) women had natural menopause; 2524 of which occurred among those under 45 years. Mean age of natural menopause for these women was 50 (SD ± 4.0 years). There were 3636 women remaining eligible for natural menopause at the end of study follow-up (i.e. the 2013 questionnaire return date).

The association between recent rotating night shift work and onset of natural menopause is presented in . Age and multivariable-adjusted models indicate a moderately increased risk of earlier menopause for women with ≥ 10 months of rotating night shift work in the previous 2-year interval. The point estimate was largest for women who had worked 10–19 months of rotating night shifts (multivariable-adjusted (MV) HR: 1.11, 95% CI: 1.01–1.21), and was similar to that for ≥ 20 months (MV HR: 1.09, 95% CI: 1.02–1.16). This highest exposure level was more strongly associated with earlier menopause for women younger than 45 years (MV HR: 1.25; 95% CI: 1.08–1.46).

Table II. Adjusted hazard ratios (HRs) and 95% CIs of natural menopause by months of rotating night shifts worked during the prior 2 years among 80 840 women of the Nurses' Health Study 2, with prospective follow-up from 1991 through 2013.

Months of rotating night shift work in past 2 years	Mean age at menopause (years, SD)	Number of Events	Age-adjusted HR (95% CI)	Multivariable-adjusted HR* (95% CI)	Multivariable-adjusted HR ^a (95% CI)	Multivariable-adjusted HR ^b (95% CI)
Overall cohort						
None	49.9 (3.8)	24 731	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
1–9	48.5 (4.4)	1187	1.09 (1.03–1.16)	1.06 (1.00–1.12)	1.05 (0.99–1.12)	1.06 (1.00–1.12)
10–19	48.1 (5.0)	483	1.12 (1.03–1.23)	1.11 (1.01–1.21)	1.10 (1.00–1.20)	1.10 (1.01–1.21)
≥ 20	48.5 (4.5)	1055	1.10 (1.04–1.17)	1.09 (1.02–1.16)	1.08 (1.02–1.15)	1.09 (1.02–1.16)
Subcohort censored at younger than 45 years						
None	41.4 (2.8)	2066	1.0 (ref.)	1.0 (ref.)	NA ^c	NA ^d
1–9	40.5 (3.6)	182	1.04 (0.90–1.21)	0.96 (0.83–1.12)		
10–19	39.9 (3.9)	90	1.23 (1.00–1.52)	1.13 (0.91–1.39)		
≥ 20	40.9 (2.9)	186	1.37 (1.18–1.59)	1.25 (1.08–1.46)		
Subcohort censored at 45 years or older						
None	50.7 (2.9)	22 665	1.0 (ref.)	1.0 (ref.)	NA ^c	NA ^d
1–9	50.0 (2.7)	1005	1.10 (1.03–1.17)	1.07 (1.00–1.14)		
10–19	49.9 (2.9)	393	1.10 (1.00–1.22)	1.09 (0.99–1.21)		
≥ 20	50.1 (2.9)	869	1.06 (0.99–1.13)	1.05 (0.99–1.13)		

*Hazard ratios adjusted for age, smoking status (never, past or current smoker), age at first birth and parity combined (nulliparous; age at first birth <24, 1–2 children; age at first birth 24–29, 1–2 children; age at first birth >29, 1–2 children; age at first birth <23, >2 children; age at first birth 24–29, >3 children; age at first birth >29, >2 children), body mass index (<18.5, 18.5 to 20, >20–22.5, >22.5–25, >25–30 and >30 kg/m²), duration of oral contraceptive use (0, 1–23, 24–47, 48–71, 72–95, 96–119 and >120 months), duration breast fed (never, ≤ 1 y, >1 y), alcohol consumption (0, >0–1, >1–4, >4–8, >8–12, >12 g/wk), physical activity (≤ 3 , >3–9, >9–19, >19–27, >27–42, >42 metabolic equivalent tasks/wk), age at menarche (≤ 9 , 10, 11, 12, 13, 14, 15, ≥ 16 y) and sleep in 24 h (≤ 4 , 5, 6, 7, 8, ≥ 9 h).

^aAdditional adjustment for years worked rotating shifts prior to 1989.

^bAdditional adjustment for cumulative rotating shift work over follow-up.

^cAdditional adjustment for year worked rotating shifts prior to 1989 not shown for age-stratified analyses.

^dAdditional adjustment for cumulative rotating shift work over follow-up not shown for age-stratified analyses.

shows the association between lifelong rotating night shift exposure and natural menopause. Working 11–20 years conveyed a slightly higher risk of earlier menopause compared to never having worked rotating night shifts, though the corresponding multivariable-adjusted effect estimate had a lower bound of one (HR: 1.06; 95% CI: 1.00–1.11). The point estimate was similar for the smaller number of women who worked >20 years of rotating night shifts (HR: 1.08; 95% CI: 0.95–1.22). Younger women (i.e. those <45 years) working more than 10 years of rotating shifts had an increased risk of earlier menopause. Working 11–20, and >20, years of rotating shifts was associated with a 22% (MV HR: 1.22; 95% CI: 1.03–1.44) and 73% (MV HR: 1.73; 95% CI: 0.90–3.35) increased risk, respectively, though only nine women in the highest exposure category under the age of 45 had the outcome.

Table III. Adjusted hazard ratios (HRs) and 95% CIs of natural menopause by lifetime cumulative history of rotating night shift work among 80 840 women of the Nurses' Health Study 2, with prospective follow-up from 1991 through 2013.

Years of cumulative rotating night shift work	Mean age at menopause (years, SD)	Number of events	Age-adjusted HR (95% CI)	Multivariable-adjusted HR* (95% CI)
Overall cohort				
None	49.8 (3.9)	8292	1.0 (ref.)	1.0 (ref.)
0–5	49.7 (4.0)	13 452	1.02 (0.99–1.05)	1.02 (0.99–1.04)
5–10	49.7 (4.0)	3429	1.05 (1.01–1.09)	1.03 (0.99–1.07)
10–15	49.8 (3.8)	1454	1.09 (1.03–1.16)	1.06 (1.00–1.12)
15–20	49.9 (3.8)	580	1.09 (1.00–1.19)	1.04 (0.96–1.14)
>20	50.8 (3.1)	249	1.08 (0.95–1.22)	1.02 (0.90–1.16)
Subcohort censored at younger than 45 years				
None	41.3 (3.0)	727	1.0 (ref.)	1.0 (ref.)
0–5	41.2 (3.0)	1274	1.08 (0.99–1.18)	1.07 (0.98–1.18)
5–10	41.3 (3.0)	333	1.13 (1.00–1.29)	1.10 (0.96–1.25)
10–15	41.4 (2.5)	127	1.24 (1.02–1.49)	1.13 (0.93–1.36)
15–20	41.8 (2.0)	54	1.72 (1.30–2.26)	1.51 (1.14–2.00)
>20	43.0 (1.0)	9	2.10 (1.09–4.05)	1.73 (0.90–3.35)
Subcohort censored at 45 years or older				
None	50.6 (2.9)	7565	1.0 (ref.)	1.0 (ref.)
0–5	50.6 (2.9)	12 178	1.01 (0.98–1.04)	1.01 (0.98–1.04)
5–10	50.6 (2.8)	3096	1.02 (1.00–1.08)	1.02 (0.98–1.06)
10–15	50.6 (2.8)	1327	1.08 (1.02–1.12)	1.06 (1.00–1.12)
15–20	50.7 (2.9)	526	1.05 (0.96–1.26)	1.01 (0.93–1.11)
>20	51.1 (2.7)	240	1.06 (0.93–1.38)	1.01 (0.89–1.15)

*Hazard ratios adjusted for age, smoking status (never, past or current smoker), age at first birth and parity combined (nulliparous; age at first birth <24, 1–2 children; age at first birth 24–29, 1–2 children; age at first birth >29, 1–2 children; age at first birth <23, >2 children; age at first birth 24–29, >3 children; age at first birth >29, >2 children), body mass index (<18.5, 18.5–20, >20–22.5, >22.5–25, >25–30 and >30 kg/m²), duration of oral contraceptive use (0, 1–23, 24–47, 48–71, 72–95, 96–119 and >120 months), duration breast fed (never, ≤1 y, >1 y), alcohol consumption (0, >0–1, >1–4, >4–8, >8–12, >12 g/wk), physical activity (≤3, >3–9, >9–19, >19–27, >27 to 42, >42 metabolic equivalent tasks/wk), age at menarche (≤9, 10, 11, 12, 13, 14, 15, ≥16 y) and sleep in 24 h (≤4, 5, 6, 7, 8, ≥9 h).

Excluding women who had diabetes prior to baseline and adjusting multivariable models using pack-years instead of smoking status did not materially affect results.

Multivariable-adjusted plotted cumulative probabilities of remaining premenopausal demonstrate that women 45 years and younger who worked ≥20 months during the prior 2-year interval were more likely to experience menopause earlier relative to those who worked none (Figure 1).

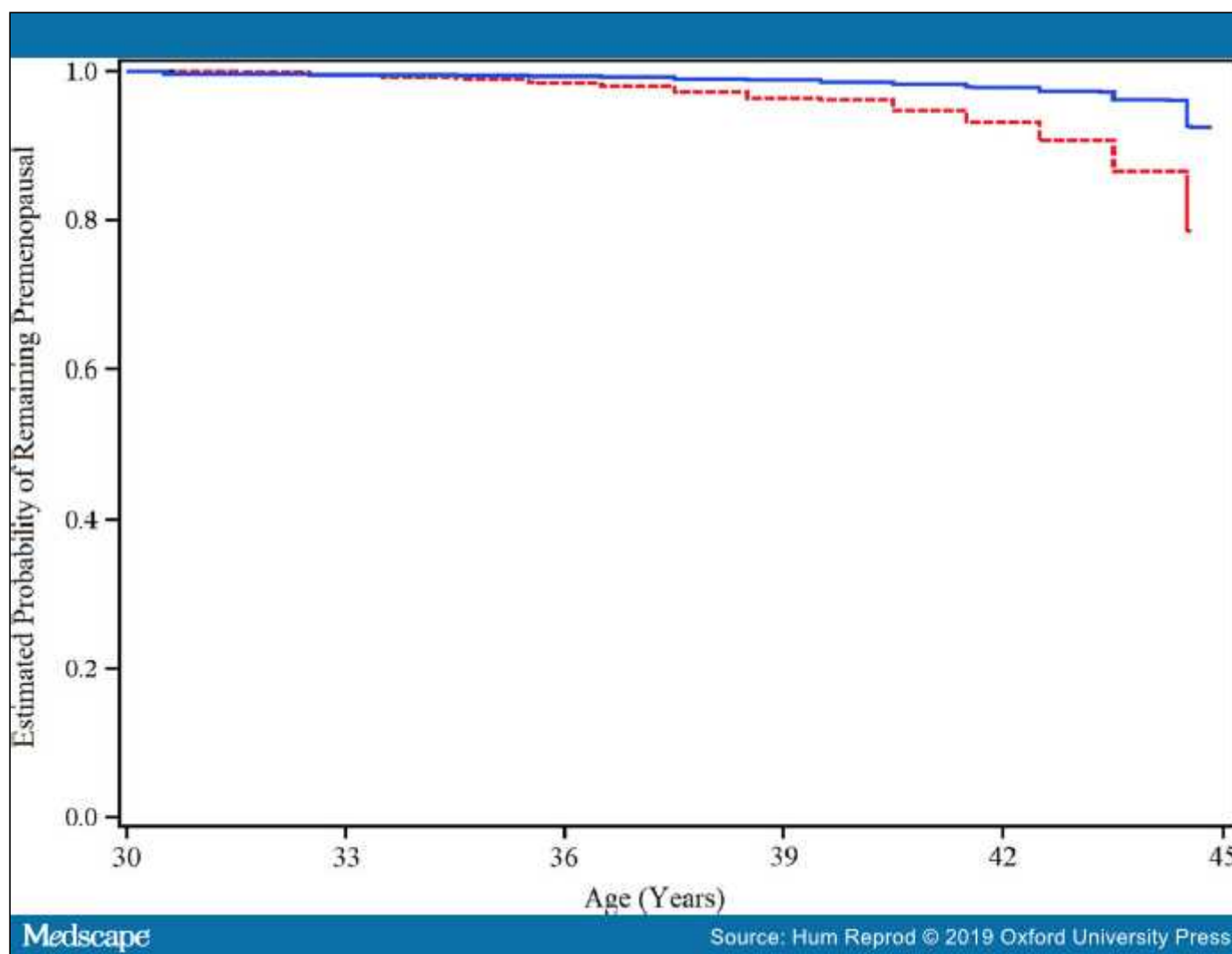


Figure 1.

Multivariable-adjusted cumulative probability of remaining premenopausal until 45 years of age across women who never worked rotating night shifts (solid blue line), and those who worked 20 or more months of rotating night shifts (hashed red line), during the prior 2 years. Adjusted for age, smoking status (never, past or current smoker), age at first birth and parity combined (nulliparous; age at first birth <24, 1–2 children; age at first birth 24–29, 1–2 children; age at first birth >29, 1–2 children; age at first birth <23, >2 children; age at first birth 24–29, >3 children; age at first birth >29, >2 children), body mass index (<18.5, 18.5–20, >20–22.5, >22.5–25, >25–30 and >30 kg/m²), duration of oral contraceptive use (0, 1–23, 24–47, 48–71, 72–95, 96–119 and >120 months), duration breast fed (never, ≤1 y, >1 y), alcohol consumption (0, >0–1, >1–4, >4–8, >8–12, >12 g/wk), physical activity (≤3, >3–9, >9–19, >19–27, >27–42, >42 metabolic equivalent tasks/wk), age at menarche (≤9, 10, 11, 12, 13, 14, 15, ≥16 y) and sleep in 24 h (≤4, 5, 6, 7, 8, ≥9 h).

Discussion

This work denotes the first prospective investigation of the association of night work with menopausal timing. Due to the complexity of central reproductive regulation, competing mechanisms support that women working more rotating night shifts over their reproductive lifetime could experience either delayed or advanced menopausal onset. Our findings suggest that women who work rotating night shifts are at increased risk of earlier menopause. Overall, a modest effect was observed for those who worked 10 or more months during the previous 2 years. Risk of earlier menopause was more pronounced for women under 45 years, for night work measured as both recent and lifelong exposures. This suggests that risk of early ovarian failure may be exacerbated by working rotating night shifts with sufficient frequency.

The increased risk of earlier menopause among rotating night shift workers does not support a net stimulatory effect of circadian disruption on ovulation. The hypothesis that circadian disruption may delay menopause via suppression of nocturnal melatonin secretion is given credence by indications of seasonal variation in human fertility. Higher conception rates have been observed during the summer in more seasonally photoperiod-diverse environments (Rojansky *et al.*, 1992) or by latitude (Batschelet *et al.*, 1973) corresponding to seasonal variation in nocturnal melatonin (Kauppila *et al.*, 1987). Mechanistic evidence for melatonin as a neuroendocrine regulator of fertility is found in seasonally breeding animal models that, while not fully elucidated, have been long acknowledged (Revel *et al.*, 2009; Dardente, 2012). It remains unclear

whether human reproductive function is materially subject to melatonin-mediated photoperiodic regulation analogous to those of seasonal breeders (Bronson, 2004).

Our findings suggest an adverse impact of circadian disruption on ovulation. Whether this is mediated by nocturnal melatonin suppression is uncertain. It is becoming recognized that coordinated timing of clock gene expression across central and peripheral sites is crucial for maintaining physiological function. Out of phase expression caused by changing environmental cues, the strongest of which is the diurnal light cycle, can jeopardize these processes. The importance of coordinated timing across the hypothalamic pituitary ovarian axis (HPOA) in ovulation is exemplified in mice by observation of clock gene regulation of both the preovulatory luteinizing hormone surge in gonadotrophs (Chu *et al.*, 2013) and time-dependent receptor mediated sensitivity to this hormone by theca cells (Mereness *et al.*, 2016), which trigger their differentiation into luteinized cells of the corpus luteum (Young and McNeilly, 2010). Shorter or more irregular menstrual cycles in night shift workers (Chung *et al.*, 2005; Su *et al.*, 2008; Lawson *et al.*, 2011; Wang *et al.*, 2016) underscore the relevance of clock-regulated processes in human ovulation and the potential dysfunction imparted by out of phase circadian stimuli.

The observation that women predisposed to early menopause may experience accelerated ovarian failure due to working sufficiently frequent rotating night shifts is novel. The major determinant of menopausal timing is the remaining number of viable candidate follicles from birth (Gougeon, 1996). Until half way through the reproductive lifetime, atresia is the most common fate of these follicles (McGee and Hsueh, 2000); however, we have no reason to suspect women with a lower baseline follicle count, or greater predisposition to atresia, would self-select rotating night work. The elevated risk of earlier menopause for women under 45 years who worked 10 or more months of recent rotating night shifts during the previous 2 years suggests that the most influential exposure window begins once the menopausal cascade is underway.

It has been suggested that melatonin may act as an anti-apoptotic agent within the follicle through receptor mediated signaling or directly as an antioxidant (Tamura *et al.*, 2012). The typically higher levels of melatonin in follicular fluid may be diminished in women who work frequent rotating night shifts due to compromised nocturnal pineal production, exacerbating follicle depletion with sufficient night work exposure.

Psychological stress has been associated with higher levels of oxidative stress and markers of declining ovarian reserve in premenopausal women (Epel *et al.*, 2004; Bleil *et al.*, 2012), potentially contributing to earlier menopause. More generally, an effect of stress on central reproductive signaling is found in animal models demonstrating altered pituitary gonadotropin secretion in response to cortisol infusion (Pierce *et al.*, 2009). Glucocorticoids, the levels of which become elevated in response to stress, are thought to interact with the HPOA at multiple sites, with potential adverse outcomes for fertility and ovulation (Whirledge and Cidlowski, 2010). It may be that working rotating nights can be chronically stress-inducing, making it difficult to separate independent effects of circadian disruption and the stress response on menopausal onset when using shift work as a surrogate exposure. Support for elevated stress in night workers can be found in studies that have observed alterations in cortisol profiles (Weibel *et al.*, 1996) or truncations of the cortisol quiescent period in circadian phase-advanced volunteers (Caufriez *et al.*, 2002; Griefahn *et al.*, 2006).

Cassou *et al.* (2007) observed that both currently working 'high-strain jobs' and working prior jobs involving 'difficult schedules' were associated with earlier menopause. Given evidence that nurses are commonly called upon to work long shifts, it is plausible that a substantial proportion of participants in the NHS2 may have met these criteria, though these were not clearly identifiable given the available data. The observation that women may work fewer rotating night shifts with increasing age may be explained in part by changes in chronotype, which tend to shift more towards morning type with age (Fischer *et al.*, 2017): women with this chronotype tend to find intermittent night shifts more strenuous (van de Ven *et al.*, 2016). As such, in addition to choosing more socially normative schedules afforded by occupational seniority, some women may avoid rotating night work as they approached menopause due to reduced biological tolerance. Whether the higher risk of early menopause among those working rotating night shift schedules observed in this study was exacerbated by chronotype-related tolerance is uncertain. While the NHS 2 did capture morning-evening preference on the 2009 questionnaire, 29% of the baseline cohort did not complete this item and data collection done at this time may have no longer reflected chronotype at time of rotating shift work exposure effect for the oldest subgroup. Further, stratifying the sample by the 2009 morning vs evening preference did not materially change findings (data not shown).

First-reported age at menopause in the NHS1 has been validated as being reliable, at least insofar as remaining fairly consistent on the subsequent biennial questionnaire for most women (Colditz *et al.*, 1987). Repeated questionnaire assessments of menopausal status also served to decrease misclassification. The potential for increased recall error with more temporally distant reporting (den Tonkelaar, 1997) further supports our use of first-reported age to define the timing of natural menopause. Despite indications that self-reported menopausal age in the NHS2 is of relatively high validity, some misclassification may have arisen due to the defining criteria. Unlike the World Health Organization definition of menopause which requires periods to have ceased for 12 months (WHO Scientific Group on Research on the Menopause in the 1990s, 1994), the NHS2 definition is limited to querying whether a woman's periods had ceased.

In addition to the novelty of this research, a key strength is the study power afforded by the NHS2, the largest cohort of female shift-workers of its kind. Despite this, power was limited for analyses restricted to women right-censored by 45 years as <10% of eligible women reported natural menopause by this age. A limitation is that rotating night shift schedule duration comprised the only routinely collected data on night work in the NHS2. The potential for misclassification due to not having more detailed shift schedule information has been discussed in previous work using NHS2 data to study rotating night work effects on reproduction-linked outcomes (Schernhammer *et al.*, 2006; Lawson *et al.*, 2011). Nevertheless, the NHS2 remains one of the only sizable cohorts in existence that collects longitudinal data on night work and potential confounding

factors. The quality of the self-reported data has been previously validated for many of the items captured on the ongoing biennial questionnaires, such as self-reported menopausal status (Colditz *et al.*, 1987).

Conclusion

Our findings suggest that premenopausal women who exceed a threshold of rotating night shift work may be at risk of moderately accelerated menopausal onset, an effect that is most pronounced among younger women potentially at elevated risk for early ovarian failure. So far, we can only speculate the degree to which disruptive circadian stimuli, through cascade effects on central and peripheral HPOA targets, including nocturnal melatonin suppression, may play a role. Further study of the interplay between circadian signaling and biological mediators of stress among shift workers, in addition to validation of this finding among studies using more detailed shift schedule data is recommended. A suggested starting point is that future occupational cohorts capture measures of 'shift system' and 'shift intensity' to supplement cumulative night work measures exemplified by the main exposures examined in this study, as recommended by the International Agency for Research on Cancer in their investigation of the carcinogenic potential of night work (Stevens *et al.*, 2011).

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