Annals of Epidemiology 25 (2015) 723–729

Contents lists available at ScienceDirect

Annals of Epidemiology

journal homepage: www.annalsofepidemiology.org

Original article

Folic acid supplement use and menstrual cycle characteristics: a cross-sectional study of Danish pregnancy planners

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ARTICLE INFO

Article history:
Received 7 October 2014
Accepted 17 May 2015
Available online 4 June 2015

Keywords:
Folic acid
Menstrual cycle characteristics
Vitamin supplementation
Preconceptional supplements

ABSTRACT

Purpose: To examine the association between folic acid (FA) supplementation obtained through either single FA tablets or multivitamins (MVs) and menstrual cycle characteristics among 5386 women aged 18–40 years, enrolled in an Internet-based study of Danish women attempting pregnancy during 2007–2011.
Methods: In a cross-sectional study, we used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of FA supplementation with menstrual cycle regularity; short (<27 days), long (30–33 days), and very long (≥34 days) cycle lengths; and duration and intensity of menstrual bleeding.
Results: Compared with nonuse, FA supplementation was associated with reduced odds of short cycle length (OR = 0.80, 95% CI: 0.68–0.94) and a trend toward increased odds of very long cycle length (OR = 1.21, 95% CI: 0.87–1.68) compared with cycle length of 27–29 days. The inverse association with short cycle length was stronger among 18- to 30-year-old women (OR = 0.68, 95% CI: 0.53–0.87), nulliparous women (OR = 0.66, 95% CI: 0.52–0.84), and women who used both FA and MVs (OR = 0.75, 95% CI: 0.60–0.95). We found no clear association between FA supplementation and cycle regularity and duration and intensity of menstrual bleeding.
Conclusions: FA supplementation was inversely associated with short menstrual cycle length. This association was strongest among women aged 18–30 years, nulliparous women, and women who used both FA and MVs.

Introduction

The menstrual cycle is mediated by endogenous hormones produced by feedback loops of the hypothalamic-pituitary-ovarian axis [1]. Previous studies have associated cycle irregularity, [2,3] and both long [2,3] and short [3–5] cycle length with reduced ability to conceive. However, the causes of cycle variability are incompletely understood [6].

Epidemiologic follow-up and cross-sectional studies have reported associations between menstrual cycle function and various reproductive [6–8] and lifestyle [6,7,9–12] factors. The most consistent predictor of cycle length is age [7], as cycles become shorter when women get older [13]. Other identified predictors of short cycle length, such as smoking, [7,8,10,14] alcohol consumption [10], and caffeine consumption [15] are modifiable behaviors that may affect the hormonal balance. Another modifiable behavior closely related to planning a pregnancy is preconception supplementation with 400-μg folic acid (FA), taken either as FA tablets or in multivitamin supplements (MVs) [16]. Folate status may play an essential role in ovulatory function, possibly through the metabolism of homocysteine [17–19]. Cross-sectional studies of women undergoing assisted reproduction have an associated elevated homocysteine concentration in the follicular fluid with poor oocyte maturity [20,21], whereas FA supplementation has been shown to increase folate concentration and decrease homocysteine concentration in the microenvironment of the maturing oocyte [21,22]. One study also suggested that women who were homozygous or heterozygous for a common gene mutation that led to decreased 5,10-methylenetetrahydrofolate reductase activity (and the

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http://dx.doi.org/10.1016/j.annepidem.2015.05.008
1047-2797/

subsequent accumulation of homocysteine [23,24]) exhibited reduced ovarian responsiveness to follicle-stimulation hormone and produced fewer oocytes [25]. Furthermore, two prospective follow-up studies of women with no history of infertility respectively showed that regular use of MVs containing FA decreased the risk of ovulatory infertility [26] and a diet high in synthetic FA reduced the risk of anovulatory cycles [27].

To the extent that FA supplementation, obtained through either single FA supplements or MVs, improves hormonal balance and follicular development, it may enhance menstrual cycle function. Two randomized trials reported improved menstrual cycle function among users of MVs (including 400- [28] and 800-μg FA [29]) either with [28] or without a history of fertility problems [29]. In the present study, we used cross-sectional data from women enrolled in a prospective cohort study of pregnancy planners to examine the association between FA supplementation obtained through single FA tablets or MVs and menstrual cycle regularity, cycle length, as well as duration and intensity of menstrual flow.

Methods

Study design

We performed a cross-sectional analysis of baseline data from the Danish Pregnancy Planning Study (Snart-Gravid), an Internet-based prospective cohort study of women planning a pregnancy between 2007 and 2011. Recruitment methods have been described previously [30–32]. Before enrollment, participants read a consent form and completed an online screening questionnaire to confirm eligibility. Participants provided a valid e-mail address and their Civil Personal Registration number. Eligible women were invited to complete an Internet-based baseline questionnaire and bimonthly follow-up questionnaires for 12 months or until conception occurred. The baseline questionnaire collected information on sociodemographic factors, reproductive and medical history, and lifestyle behaviors. Participants were initially randomized to receive either a short- or a long-form baseline questionnaire, with some questions asked of only 50% of the cohort during the first 6 months of enrollment. Completion rates and missing data proportions were similar for both questionnaire versions [31].

Study population and study period

Eligible women were Danish residents aged 18–40 years, in a stable relationship with a male partner, attempting to conceive for no more than 12 months, and not receiving fertility treatment at study entry. From June 2007 to August 2011, 5387 women enrolled in the study. One woman who was already pregnant (14 weeks) was excluded. In total, 5386 eligible women enrolled in the study.

Assessment of menstrual cycle characteristics

The menstrual cycle characteristics examined in the present study were cycle regularity, cycle length, duration of menstrual bleeding, and intensity of menstrual bleeding. Women who responded "yes" to the question "Are your menstrual periods regular?" were considered to have regular cycles.

Among the 4041 women with regular cycles, we assessed cycle length, defined as "the number of days from the first day of a menstrual period to the first day of the next menstrual period" in categories of short (<27 days), normal (27–29 days), long (30–33 days), and very long (≥34 days). Duration and intensity of menstrual bleeding were assessed among women with regular cycles by means of the questions, "How many days does your period usually flow (bleeding not spotting)?" in categories of short (<3 days), normal (3–4 days), long (5–6 days), and very long (>6 days) and "How would you classify the total amount of your menstrual flow?" in categories of light (≤10 pads or tampons per menstrual cycle), moderate (11–20 pads or tampons per menstrual cycle), heavy (21–30 pads or tampons per menstrual cycle), and very heavy (>30 pads or tampons per menstrual cycle).

Assessment of preconception FA and MV use

In the baseline questionnaire, respondents were asked, "Do you take vitamins on a regular basis - daily or almost every day?", "How long have you been taking vitamins on a regular basis - less than one year, 1-5 years, more than 5 years and 'don't know'?", and "Which of the following vitamins or minerals do you take on a regular basis - MVs, vitamin A, beta-carotene, vitamin B, vitamin C, vitamin D, vitamin E, FA, calcium, magnesium, selenium and 'other'?" Participants who reported "multivitamin" or wrote the name of an MV product were classified as "MV users." Similarly, participants who reported "folic acid" or wrote "folate" were classified as "FA users." Most MVs marketed in Denmark contain 400 μg of FA, especially those made for use during pregnancy. Therefore, we created a single binary exposure variable defined as "FA supplementation," which was set to 1 for women who were FA users, MV users, or both. For women who used single vitamin or mineral supplements other than FA and women who did not take any dietary supplements, the exposure variable "FA supplementation" was set to 0 and was defined as "nonuse."

Assessment of covariates

From the baseline questionnaire, we obtained data on previously recognized correlates of menstrual cycle characteristics or correlates of FA or MV supplement use, including age, education, intercourse frequency, participation in the national screening program for cervical cancer (pap smear) during the last 3 years, history of miscarriage, parity, smoking, alcohol use, body mass index (BMI), physical activity level, caffeine intake, and last method of contraception. We calculated BMI from self-reported weight and height (kilogram per square meter). Total metabolic equivalents (METs) were estimated by summing the METs from moderate physical activity (hours per week multiplied by 3.5) and vigorous physical activity (hours per week multiplied by 7.0) [33].

Data analysis

We examined the association between FA supplementation and cycle regularity using logistic regression to estimate odds ratios (ORs) with 95% confidence intervals (CIs). For the analysis of cycle length and duration and intensity of menstrual bleeding, we restricted the analysis to women with regular cycles. We used polytomous logistic regression to estimate ORs with 95% CIs for the association of FA supplementation with short (<27 days), long (30–33 days), and very long (≥34 days) cycle length, compared with cycle length of 27–29 days. We also estimated ORs with 95% CIs for the association of FA supplementation with short (<3 days), long (5–6 days), and very long (>6 days) duration of menstrual bleeding, compared with normal (3–4 days) duration of bleeding, and with light (≤10 pads or tampons/menstrual cycle), heavy (21-30 pads or tampons/menstrual cycle), and very heavy (>30 pads or tampons/menstrual cycle) intensity of menstrual bleeding, compared with moderate (11-20 pads or tampons/menstrual cycle) intensity of bleeding. The multivariate analyses were adjusted for age (<25, 25–29, 30–34, and ≥35 years), parity (parous vs. nulliparous), previous miscarriage (yes vs. no), BMI (<18.5, 18.5–24.9, 25–29, 30–34.9, and ≥35 kg/m^2), smoking status

(current smoker, occasional smoker, former smoker, and never smoker), alcohol intake (none, 1–3,4–7, 8–14, and ≥15 drinks/wk), caffeine intake (<100, 100–199, 200–299, and ≥300 mg/d), physical activity (<10, 11–19, 20–39, and ≥40 METs/wk), and last method of contraception (barrier methods, oral contraceptives, and other methods). For the analyses of duration of menstrual flow, we also adjusted for intensity of flow and vice versa. To eliminate the effect of hormonal contraceptives on menstrual cycle function, we also evaluated the associations after restricting the study population to women who reported a nonhormonal method of last contraception. In a subanalysis, we stratified the data according to parity (parous and nulliparous) and age (18–30 and 31–40 years) at study entry to evaluate potential effect-measure modification. For the stratified analyses of duration of menstrual flow, we adjusted for pack-years of smoking (never smoked, <5, 5–9, and ≥10 pack-years) instead of current smoking status because of too few women in the categories. For the purpose of a subanalysis, we also created three mutually exclusive categories of vitamin supplement use (FA exclusively, MV exclusively, and FA and MV) to address their independent relation with menstrual cycle regularity and cycle length.

Because 1540 (28.6%) participants were randomized to receive the short-form baseline questionnaire, they did not receive the questions about the intensity of menstrual bleeding. Therefore, the proportion of missing data for the intensity of menstrual bleeding was 28.8%, but those missing this information were a random subset of enrollees by design. One hundred ninety-seven (3.7%) women did not answer the initial vitamin question. The amount of missing data for covariates and menstrual cycle characteristics ranged between 0.1% and 2.9%. We addressed missing data by using multiple imputation [34,35]. We generated five complete data sets, which were analyzed, and the results were combined by the rules of Rubin [36]. The absolute numbers of FA supplement users and nonusers listed in the tables were based on the first imputed data set. Stata statistical software (version 11.2, Stata Corp., College Station, TX) was used for all analyses.

Results

Overall, 3344 (62.1%) women used FA supplements exclusively (7.7%), MV supplements exclusively (20.4%), or both (34.0%). Another 2042 (37.9%) women did not use any dietary supplements (36.4%) or used single vitamins or minerals other than FA (1.5%). Among users, 37.7% took supplements for 1 year or more. Characteristics of the study population according to FA supplementation are listed in Table 1. Users tended to be older, have higher education, be nonsmokers, be physically active, consume less alcohol and caffeine, and have lower BMI. Users were also more likely to have greater intercourse frequency, a history of miscarriage, regular pap smears, and to have used barrier methods as their last method of contraception.

Association between FA supplementation and cycle regularity

A total of 1345 (25.0%) women reported irregular periods. Overall, we found little association between FA supplementation and cycle regularity (adjusted OR = 0.98, 95% CI: 0.86–1.12; Table 2). Compared with nonuse, FA supplementation was associated with slightly reduced odds of having irregular cycles among parous women (adjusted OR = 0.83, 95% CI: 0.65–1.04). For the relation between the use of FA exclusively, MVs exclusively, and FA and MVs and cycle regularity, the respective adjusted ORs (95% CI) were 1.10 (0.89–1.44), 1.09 (0.65–1.04), and 0.89 (0.76–1.03).

Association between FA supplementation and cycle length

The mean and median cycle lengths in our sample were 28.6 and 28 days (range: 14–55 days). Among the 4041 regularly cycling women, 597 (14.8%) reported cycles of less than 27 days, 2214 (54.8%) reported cycles of 27–29 days, 1056 (26.1%) reported cycles of 30–33 days, and 174 (4.3%) reported cycles of 34 days or more (Table 3). Twenty women (0.5%) reported cycles of ≥40 days. Overall, compared with nonuse, FA supplementation was associated with reduced odds of short cycle length (<27 days; adjusted OR = 0.80, 95% CI: 0.68–0.94) and a trend toward increased odds of very long cycle length (≥34 days; adjusted OR = 1.21, 95% CI: 0.87–1.68). The overall associations were consistent after restricting the analyses to women who reported a nonhormonal method of last contraception ($n = 2547$; adjusted OR = 0.78, 95% CI: 0.64–0.96 for short cycle length and adjusted OR = 1.19, 95% CI: 0.86–1.66 for very long cycle length). In the stratified analyses, we found a stronger inverse association between FA supplementation and short cycle length among nulliparous women (adjusted OR = 0.66, 95% CI: 0.52–0.84). The adjusted OR was 1.12 (95% CI: 0.79–1.57) among parous women. Also, we found a stronger association between FA supplementation and short cycle length among women aged 18–30 years (adjusted OR = 0.68, 95% CI: 0.53–0.87) than among women aged 31–40 years (adjusted OR = 1.02, 95% CI: 0.73–1.42). Compared with nonusers, FA users aged 31–40 years had increased odds of having very long cycle length (adjusted OR = 1.63, 95% CI: 0.74–3.61), whereas FA users aged 18–31 years only had slightly increased odds of having very long cycle length (adjusted OR = 1.09, 95% CI: 0.75–1.59). Similarly, parous FA users tended to have increased odds of having very long cycle length (adjusted OR = 1.78, 95% CI: 0.87–3.64) compared with nulliparous FA users (adjusted OR = 1.08, 95% CI: 0.73–1.59). In secondary analyses, we found an even stronger association between FA supplementation and cycle length of less than 25 days (adjusted OR = 0.72, 95% CI: 0.49–1.05). Adjusted OR with 95% CI of having cycle length of 25–26 days, 30–31 days, 32–33 days, and ≥34 days were 0.85 (0.68–1.06), 0.94 (0.80–1.10), 0.99 (0.74–1.33), and 1.21 (0.87–1.68), respectively, compared with cycle length of 27–29 days (Supplementary Table 1). Finally, for the relation

Table 1
Baseline characteristics of 5386 women according to FA or MV supplement use

Characteristic	Vitamin supplement use	
	FA supplement use	Nonuse*
Number of women	3344 (62.1%)	2042 (37.9%)
Age, y (mean)	28.6	27.9
BMI, kg/m^2 (mean)	24.1	24.7
Pap smear, once or more during the last 3 y (%)†	83.3	73.2
Higher education, >4 y (%)	24.2	17.4
Parous, ever had live birth (%)	33.2	33.4
Previous miscarriage, yes (%)	11.7	9.5
Intercourse frequency, ≥1 times/wk (%)	85.3	80.8
Caffeine intake, ≥300 mg/d (%)	11.5	14.2
Smoking status (%)		
Current smoker	9.9	21.9
Occasional smoker	5.0	6.9
Former smoker	22.4	19.6
Never smoker	62.7	51.6
Pack-years of smoking (mean)	5.2	5.9
Alcohol intake, drinks/wk (mean)	2.5	3.1
Physical activity, h/wk (mean)	25.0	23.8
Last method of contraception (%)		
Hormonal contraceptives	52.2	53.5
Barrier methods	28.0	24.2

* Nonuse includes no supplement use (36.4%) and the use of single vitamin and/or mineral supplements other than FA acid (1.5%).
† Attending the national screening program for cervical cancer (pap smear).

Table 2
Crude and adjusted ORs with 95% CIs for irregular cycles compared with regular cycles, by parity, age, and use of FA supplementation ($N = 5386$)

Supplement use			All N (%)	Regular cycles (ref.) n	Irregular cycles	
					n	OR (95% CI)
Overall	FA use	Crude	3344 (62.1)	2516	828	0.97 (0.85–1.10)
		Adjusted*				0.98 (0.86–1.12)
	Nonuse		2042 (37.9)	1525	517	1 (Ref.)
Parous	FA use	Crude	1111 (20.6)	871	240	0.83 (0.66–1.04)
		Adjusted†				0.83 (0.65–1.04)
	Nonuse		682 (12.7)	514	168	1 (Ref.)
Nulliparous	FA use	Crude	2233 (41.5)	1645	588	1.04 (0.89–1.21)
		Adjusted†				1.05 (0.90–1.23)
	Nonuse		1360 (25.2)	1011	349	1 (Ref.)
18–30 y	FA use	Crude	2294 (42.6)	1661	633	1.01 (0.87–1.17)
		Adjusted‡				1.01 (0.86–1.16)
	Nonuse		1485 (27.6)	1077	408	1 (Ref.)
31–40 y	FA use	Crude	1050 (19.5)	855	195	0.93 (0.71–1.20)
		Adjusted‡				0.88 (0.67–1.14)
	Nonuse		557 (10.3)	448	109	1 (Ref.)
FA use exclusively		Crude	412 (7.7)	297	115	1.15 (0.91–1.46)
		Adjusted*				1.10 (0.87–1.44)
MV use exclusively		Crude	1101 (20.4)	805	296	1.08 (0.92–1.28)
		Adjusted*				1.09 (0.92–1.30)
FA and MV use		Crude	1831 (34.0)	1414	417	0.87 (0.75–1.01)
		Adjusted*				0.89 (0.76–1.03)

* Overall: adjusted for age, BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.
† Parity: adjusted for age, BMI, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.
‡ Age: adjusted for BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.

between the use of FA exclusively, MVs exclusively, and FA and MVs and cycle length, the respective adjusted ORs were 0.93 (95% CI: 0.62–1.38), 0.82 (95% CI: 0.67–1.01), and 0.75 (95% CI: 0.60–0.95), for short cycle length and 1.02 (95% CI: 0.52–1.97), 1.18 (95% CI: 0.75–1.84), 1.27 (95% CI: 0.89–1.82), for very long cycle length.

Association between FA supplementation and duration and intensity of menstrual bleeding

Among the 4041 regularly cycling women, 367 (9.1%) reported a menstrual flow of less than 3 days, 2158 (53.4%) reported a menstrual flow of 3–4 days, 1368 (33.9%) reported a menstrual flow of 5–6 days, and 148 (3.7%) reported a menstrual flow of more than 6 days (Table 4). Among these women, 993 (24.6%) reported "light" menstrual bleeding (≤10 pads or tampons per menstrual cycle), 2386 (59.0%) reported "moderate" menstrual bleeding (11–20 pads or tampons per menstrual cycle), 589 (14.6%) reported "heavy" bleeding (21–30 pads or tampons per menstrual cycle), and 73 (1.8%) reported "very heavy" bleeding (>30 pads or tampons per menstrual cycle; Table 5). Overall, there was no clear association between FA supplementation and duration and intensity of menstrual flow, and the estimates were imprecise. For light intensity of

Table 3
Crude and adjusted ORs with 95% CIs for cycle length of less than 27, 27–29, 30–33, and 34 days or more compared with normal cycle length (27–29 days), by FA supplementation, among women with regular cycles ($n = 4041$)

Supplement use			Cycle length (d)							
			All	27–29 (ref.)	<27		30–33		≥34	
			N (%)	n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
Overall	FA use	Crude	2516 (62.3)	1417	336	0.74 (0.61–0.90)	645	0.89 (0.77–1.04)	118	1.14 (0.82–1.60)
		Adjusted*				0.80 (0.68–0.94)		0.95 (0.82–1.11)		1.21 (0.87–1.68)
	Nonuse		1525 (37.7)	797	261	1 (ref.)	411	1 (ref.)	56	1 (ref.)
Parous	FA use	Crude	871 (21.6)	494	128	1.02 (0.74–1.43)	217	0.92 (0.71–1.19)	32	1.50 (0.76–2.98)
		Adjusted†				1.12 (0.79–1.57)		0.95 (0.72–1.25)		1.78 (0.87–3.64)
	Nonuse		514 (12.7)	289	73	1 (ref.)	140	1 (ref.)	12	1 (ref.)
Nulliparous	FA use	Crude	1645 (40.7)	923	208	0.63 (0.50–0.80)	428	0.88 (0.73–1.06)	86	1.03 (0.71–1.52)
		Adjusted†				0.66 (0.52–0.84)		0.90 (0.74–1.09)		1.08 (0.73–1.59)
	Nonuse		1011 (25.0)	508	188	1 (ref.)	271	1 (ref.)	44	1 (ref.)
18–30 y	FA use	Crude	1661 (41.1)	929	197	0.65 (0.51–0.83)	444	0.81 (0.67–0.97)	91	1.07 (0.73–1.54)
		Adjusted‡				0.68 (0.53–0.87)		0.80 (0.66–0.96)		1.09 (0.75–1.59)
	Nonuse		1077 (26.6)	530	179	1 (ref.)	321	1 (ref.)	47	1 (ref.)
31–40 y	FA use	Crude	855 (21.2)	488	139	0.93 (0.68–1.28)	201	1.21 (0.90–1.62)	27	1.60 (0.74–3.46)
		Adjusted‡				1.02 (0.73-1.42)		1.22 (0.90–1.65)		1.63 (0.74–3.61)
	Nonuse		448 (11.1)	267	82	1 (ref.)	90	1 (ref.)	9	1 (ref.)
FA use exclusively		Crude	297 (7.4)	168	45	0.84 (0.59–1.21)	70	0.83 (0.61–1.12)	14	1.13 (0.61–1.10)
		Adjusted*				0.93 (0.62–1.38)		0.85 (0.60–1.20)		1.02 (0.52–1.97)
MV use exclusively		Crude	805 (19.9)	453	108	0.74 (0.57–0.96)	207	0.91 (0.74–1.11)	37	1.10 (0.71–1.71)
		Adjusted*				0.82 (0.67–1.01)		0.95 (0.77–1.18)		1.18 (0.75–1.84)
FA and MV use		Crude	1414 (35.0)	796	183	0.72 (0.58–0.90)	368	0.90 (0.76–1.07)	37	1.17 (0.81–1.69)
		Adjusted*				0.75 (0.60–0.95)		0.97 (0.83–1.15)		1.27 (0.89–1.82)

* Overall: adjusted for age, BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.
† Parity: adjusted for age, BMI, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.
‡ Age: adjusted for BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.

Table 4
Crude and adjusted ORs with 95% CIs for duration of menstrual flow of <3, 5–6, and <6 days compared with normal duration of menstrual flow (3–4 days), by parity, age, and FA supplementation, among women with regular cycles ($n = 4041$)

Supplement use			Duration of menstrual flow (d)							
			All	3–4 (ref.)	<3		5–6		>6	
			N (%)	*n*	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Overall	FA use	Crude	2516 (62.3)	1324	234	1.10 (0.87–1.38)	859	1.06 (0.92–1.22)	99	1.27 (0.89–1.81)
		Adjusted*				1.14 (0.89–1.46)		1.04 (0.89–1.21)		1.22 (0.82–1.81)
	Nonuse		1525 (37.7)	834	133	1 (ref.)	509	1 (ref.)	49	1 (ref.)
Parous	FA use	Crude	871 (21.6)	430	61	0.98 (0.63–1.53)	332	1.07 (0.84–1.35)	48	1.54 (0.89–2.69)
		Adjusted†				1.11 (0.67–1.83)		1.05 (0.81–1.36)		1.46 (0.74–2.89)
	Nonuse		514 (12.7)	264	37	1 (ref.)	194	1 (ref.)	19	1 (ref.)
Nulliparous	FA use	Crude	1645 (40.7)	894	173	1.15 (0.87–1.50)	527	1.07 (0.90–1.28)	51	1.08 (0.68–1.72)
		Adjusted†				1.11 (0.85–1.48)		1.09 (0.91–1.32)		1.15 (0.72–1.86)
	Nonuse		1011 (25.0)	570	96	1 (ref.)	315	1 (ref.)	30	1 (ref.)
18–30 y	FA use	Crude	1661 (41.1)	861	141	1.02 (0.78–1.35)	594	1.11 (0.94–1.31)	65	1.12 (0.74–1.40)
		Adjusted‡				1.07 (0.80–1.45)		1.08 (0.90–1.30)		1.06 (0.68–1.67)
	Nonuse		1077 (26.7)	582	93	1 (ref.)	363	1 (ref.)	39	1 (ref.)
31–40 y	FA use	Crude	855 (21.2)	463	93	1.24 (0.83–1.86)	265	0.98 (0.76–1.27)	34	1.84 (0.89–3.79)
		Adjusted‡				1.30 (0.81–2.01)		0.91 (0.70–1.23)		2.07 (0.89–4.45)
	Nonuse		448 (11.0)	252	40	1 (ref.)	146	1 (ref.)	10	1 (ref.)

* Overall: adjusted for age, BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and intensity of menstrual flow.
† Parity: adjusted for age, BMI, previous spontaneous abortions, pack-years of smoking (self-reported smoking was categorized as pack-years of ever smoking, with 1 pack-year defined as smoking 20 cigarettes per day for 1 year), alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and intensity of menstrual flow.
‡ Age: adjusted for BMI, parity, previous spontaneous abortions, pack-years of smoking (self-reported smoking was categorized as pack-years of ever smoking, with 1 pack-year defined as smoking 20 cigarettes per day for 1 year), alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and intensity of menstrual flow.

flow (≤10 pads or tampons per menstrual cycle), the adjusted OR was 0.82 (95% CI: 0.66–1.02).

Discussion

Main findings

In this cross-sectional study of Danish pregnancy planners aged 18–40 years, FA supplementation was associated with reduced odds of short cycle length (<27 days) and a trend toward increased odds of very long (≥34 days) cycle length. The inverse association between FA supplementation and short cycle length was strongest among women aged 18–30 years, nulliparous women, and among women who used both FA and MVs, whereas the association between FA supplementation and very long cycle length was strongest among 31- to 40-year-old women, parous women, and among women who used both FA and MVs.

Strengths and limitations

The main limitation of our study is the cross-sectional nature of the baseline data. Information on supplement use was ascertained simultaneously with menstrual cycle characteristics. Although 38% of users in our study reported using supplements for at least 1 year, we have no information on duration of use of the specific supplements, the exact dose of FA ingested, or brand name of all FA or MV

Table 5
Crude and adjusted ORs with 95% CIs for intensity of menstrual flow of 10 or less, 21–30, and 30 pads or tampons per day compared with moderate flow (11–20 pads or tampons per day), by parity, age, and FA supplementation, among women with regular cycles ($n = 4041$)

Supplement use			Intensity of menstrual flow (pads or tampons/menstrual cycle)							
			All	11–20 (ref.)	≤10		21–30		>30	
			N (%)	*n*	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Overall	FA use	Crude	2516 (62.3)	1519	585	0.85 (0.71–1.01)	361	0.91 (0.73–1.12)	51	1.25 (0.59–2.65)
		Adjusted*				0.82 (0.66–1.02)		0.91 (0.73–1.12)		1.38 (0.64–2.98)
	Nonuse		1525 (37.7)	867	408	1 (ref.)	228	1 (ref.)	22	1 (ref.)
Parous	FA use	Crude	871 (21.6)	544	123	0.76 (0.53–1.09)	172	0.91 (0.65–1.28)	32	1.37 (0.48–3.90)
		Adjusted†				0.78 (0.52–1.16)		0.90 (0.63–1.27)		1.47 (0.46–4.73)
	Nonuse		514 (12.7)	300	96	1 (ref.)	106	1 (ref.)	12	1 (ref.)
Nulliparous	FA use	Crude	1645 (40.7)	975	462	0.88 (0.72–1.06)	189	0.90 (0.69–1.18)	19	1.09 (0.45–2.63)
		Adjusted†				0.84 (0.67–1.05)		0.91 (0.69–1.21)		1.39 (0.51–3.81)
	Nonuse		1011 (25.0)	567	312	1	122	1 (ref.)	10	1 (ref.)
18–30 y	FA use	Crude	1661 (41.1)	994	396	0.89 (0.72–1.10)	245	0.99 (0.78–1.25)	26	1.20 (0.59–2.45)
		Adjusted‡				0.90 (0.70–1.14)		1.04 (0.81–1.32)		1.47 (0.68–3.16)
	Nonuse		1077 (26.7)	617	294	1 (ref.)	153	1 (ref.)	13	1 (ref.)
31–40 y	FA use	Crude	855 (21.2)	525	189	0.76 (0.54–1.06)	116	0.74 (0.50–1.10)	25	1.28 (0.28–5.67)
		Adjusted‡				0.68 (0.46–1.01)		0.64 (0.42–0.98)		1.04 (0.20–5.39)
	Nonuse		448 (11.0)	250	114	1 (ref.)	75	1 (ref.)	9	1 (ref.)

* Overall: adjusted for age, BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and duration of menstrual flow.
† Parity: adjusted for age, BMI, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and duration of menstrual flow.
‡ Age: adjusted for BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, last contraception method used, cycle length, and duration of menstrual flow.

products. Thus, we cannot determine the exact temporal sequence between FA supplementation and menstrual cycle characteristics.

Some participants may have reported themselves as "users" simply because they were asked or were planning to begin supplementation within a short time. Therefore, some users in this study may be misclassified. In addition, MV supplements have no standard definitions, and it is uncertain whether all MVs contained FA, which may also lead to some misclassification. However, most MVs marketed in Denmark contain 400-μg FA, especially those made for use during pregnancy. We see no reason, however, why misclassification of information on vitamin use would differ between subgroups of women with different menstrual cycle characteristics or why information on cycle length would differ between FA supplement users and nonusers. Therefore, any misclassification errors in assessing supplement use should bias results toward the null.

We did not validate the accuracy of menstrual cycle length reported by participants in this study. However, a subanalysis of participants from the Snart-Gravid study population found modest agreement between menstrual cycle length reported on the baseline questionnaire and on a subsequent follow-up questionnaire (Pearson's correlation [$r = 0.60$]) [5].

Previously identified predictors of FA or MV use indicate various demographic, lifestyle, and behavioral differences between FA supplement users and nonusers, for example, users were less likely to smoke and consume alcohol [37]. Such health behaviors also may be related to menstrual cycle function which may confound the estimates [7,8,10,14]. In the regression models, we adjusted for several lifestyle factors, and it seems unlikely that an underlying health factor would meaningfully affect the associations. Furthermore, we stratified by age and parity in the analysis to evaluate effect-measure modification by factors related to age or pregnancy and childbirth. We have no information about the length of the interpregnancy interval among parous women. Thus, menstrual cycle length may be influenced by recent child birth or breast feeding, which may explain some of the differences in the estimates between parous and nulliparous women.

Because Snart-Gravid participants may have been more health conscious than women planning pregnancy in general, we may have observed a higher prevalence of FA and MV users compared with the general population of Danish pregnancy planners. However, our comparisons were made within the population of study participants (rather than between study participants and pregnancy planners who did not participate in the study), and the internal validity of the study should not be affected by differences between the study participants and the general population [38]. In addition, participants in the Snart-Gravid study were enrolled without regard to supplement use or menstrual cycle characteristics. We excluded women who had been trying to conceive for more than 12 months at study entry to decrease the risk of changes in exposures as the women did not conceive. Thus, at study entry participants had no knowledge that the association between supplement use and menstrual cycle characteristics would be examined, reducing the possibility of bias.

Interpretation

To our knowledge, only two studies assessed the association between preconception FA-containing MV supplementation and menstrual cycle characteristics among women with and without recognized fertility problems [28,29]. Dudás et al. [29] reported improved menstrual cycle regularity by means of lower variation of cycle length after supplementation with MVs that included 800-μg FA, compared with placebo among 1000 healthy women [29]. Women took supplements until conception occurred or for 12 months, allowing observation of changes in menstrual cycle characteristics during supplementation. Dudás et al. [29] found no change in cycle length during supplementation and found only little association between the use of MV supplements and the length of menstrual bleeding. In another randomized trial of 93 women who had tried unsuccessfully to conceive for 6–36 months, Westphal et al. [28] reported normalization of both short and long cycle lengths as well as increased progesterone levels after supplementation for 3 months with MVs containing 400-μg FA [28]. However, the study used no relative estimates and CIs to demonstrate the strength and precision of the association, and there was little difference between treatment groups. Still, it is difficult to compare the two trials with our study because of differences in study designs, definitions of short and long cycle length, and study populations. Our findings were also supported by Gaskins et al. [27], who reported that a diet high in synthetic FA reduced the risk of anovulatory cycles as well as higher luteal progesterone levels among women without a history of infertility.

Because most participants used FA supplements in combination with MVs, the effect attributable to FA alone could not be determined in this study. However, we found a stronger inverse association between the use of both FA and MVs and short cycle length compared with the use of FA or MVs exclusively, which may indicate a synergistic effect of FA in combination with MVs. A randomized controlled trial of 56 women undergoing ovulation induction revealed that supplementation with an MV product during ovulation increased pregnancy rates compared with supplementation with FA alone [39]. Of interest, a recent study on 232 women undergoing assisted reproduction in the Unites States found that a high intake of supplemental FA (>800 μg/d) was associated with higher rates of implantation, clinical pregnancy, and live birth, but also with a lower count of mature oocytes, compared with FA intake of less than 400 μg/d [40]. However, the U.S. study included women with high total folate intake (median intake 1778 μg/d), which may be substantially higher than in our study because of mandatory food fortification in the United States [41] and, unlike our study, included women undergoing assisted reproduction.

Conclusion

In conclusion, preconception FA supplementation was inversely associated with short menstrual cycle length. This association was strongest among women aged 18–30 years, nulliparous women, and women who used both FA and MVs.

Acknowlegdments

We are grateful to Tina Christensen for her support with data collection and media contacts.

This work was supported by the National Institute of Child Health and Human Development (R21-050264) and the Danish Medical Research Council (271-07-0338). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The Snart Gravid study was approved by the Danish Data Protection Board (2006-41-6864), and no further approval is required according to the Danish Ethical Review System. The study was approved by the Institutional Review Board at Boston University, and consent was obtained from all participants via the Internet.

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Appendix

Supplementary Table 1
Crude and adjusted ORs with 95% CIs for cycle length of less than 25, 25–26, 30–31, 32–33, and 34 days or more compared with normal cycle length (27–29 days), by FA supplementation, among women with regular cycles ($n = 4041$)

Supplement use	Cycle length (d)											
	All	27–29 (ref.)	<25		25–26		30–31		32–33		≥34	
	N (%)	*n*	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
FA use												
Crude	2516 (62.3)	1417	118	0.67 (0.48–0.94)	218	0.84 (0.68–1.03)	530	0.92 (0.79–1.07)	115	1.03 (0.78–1.35)	118	1.17 (0.86–1.58)
Adjusted*				0.72 (0.49–1.05)		0.85 (0.68–1.06)		0.94 (0.80–1.10)		0.99 (0.74–1.33)		1.21 (0.87–1.68)
Nonuse	1525 (37.7)	797	104	1 (ref.)	157	1 (ref.)	337	1 (ref.)	74	1 (ref.)	56	1 (ref.)

* Adjusted for age, BMI, parity, previous spontaneous abortions, smoking status, alcohol and caffeine intake, physical activity, and last contraception method used.