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Original Contribution

Cutaneous Melanoma and Endogenous Hormonal Factors: A Large French Prospective Study

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To assess the role of endogenous hormonal factors on melanoma, the authors conducted a prospective analysis of 91,972 French women, aged 40–65 years at inclusion into the Etude Epidémiologique auprès de femmes de l'Education Nationale (E3N) cohort. Between 1990 and 2005, 460 melanoma cases were ascertained. Relative risks and 95% confidence intervals were computed by using Cox proportional hazards regression models. Risks of melanoma were reduced in women with ≥15 years at menarche (relative risk (RR) = 0.67, 95% confidence interval (CI): 0.46, 0.97, compared with 13–14 years), irregular menstrual cycles (RR = 0.52, 95% CI: 0.31, 0.89, compared with regular cycles of 25–31 days), <48 years at natural menopause (RR = 0.70, 95% CI: 0.48, 1.02, compared with 48–51 years), and shorter ovulatory life (RR = 0.51, 95% CI: 0.28, 0.91, for <33 years compared with ≥39 years). Modest inverse associations were observed with parity, as well as number of pregnancies and miscarriages. There was no evidence of an association between melanoma risk and age at first birth or pregnancy, age at last birth, time since last birth, breastfeeding duration, age at menstruation regularity, or menopausal status. Results did not significantly differ according to ambient ultraviolet radiation dose and melanoma site or subtype. These findings from a large prospective cohort may suggest a reduced melanoma risk associated with decreased exposure to ovarian hormones.

cohort studies; hormones; melanoma; menarche; menopause; menstrual cycle; reproductive history

Abbreviations: CI, confidence interval; E3N, Etude Epidémiologique auprès de femmes de l'Education Nationale; RR, relative risk.

The incidence of cutaneous melanoma has risen considerably in Caucasian populations around the world during recent decades (1). Some authors have hypothesized a hormonal dependence of melanoma, mostly on the basis of early case reports and incidence data (2). Subsequently, several epidemiologic studies reported a reduced melanoma risk with higher parity and a higher risk with higher age at first livebirth and use of oral contraceptives (3–6). Overall, however, epidemiologic evidence for associations between endogenous hormonal factors and melanoma risk is inconclusive. Most reports have been based on case-control studies (4, 7–20) or have relied upon record linkage without adjustment for the established melanoma risk factors (6, 21, 22), and few prospective studies are available (5, 23–25). Moreover, different anatomic sites and histologic types of melanoma have been associated with distinct characteristics and exposures (26), but few studies have been able to examine the relations between melanoma and hormonal factors according to tumor site and type. The purpose of our study was to investigate the potential influence of endogenous hormonal factors on melanoma risk in women by using data from the Etude Epidémiologique auprès de femmes de l'Education Nationale (E3N), a large prospective cohort.

MATERIALS AND METHODS

The E3N cohort

The E3N is a prospective cohort involving 98,995 French women, aged 40–65 years at inclusion and insured by a national health insurance plan primarily covering teachers.

Participants were enrolled in 1989–1991 after returning an informed consent and replying to a baseline self-administered questionnaire on their lifestyle and medical history. Follow-up questionnaires were sent every 2–3 years thereafter and addressed medical events such as cancer, which were confirmed though pathology reports.

Data collection

Endogenous hormonal factors. Age at menarche was collected in the 1990 and 1992 questionnaires, while menstrual cycle length during midlife and age at menstrual cycle regularity were collected in 1992. Parity, number of pregnancies (including births, miscarriages, ectopic pregnancies, and induced abortions), age at each pregnancy, history of miscarriages, and breastfeeding status and duration for each livebirth were collected in the 1990 and 1992 questionnaires. Menopausal status and age at menopause were available at inclusion and in each follow-up questionnaire. Women were considered postmenopausal if they reported 12 consecutive months of amenorrhea, bilateral oophorectomy, use of menopausal hormone therapy, or being postmenopausal. When age at natural menopause was considered, women with artificial menopause or unknown natural/artificial menopausal status were excluded. We also considered length of ovulatory life, computed as age at natural menopause minus age at menarche.

Other factors. The baseline questionnaire collected data on education and pigmentary traits such as hair color (red, blond, chestnut, brown, black), skin complexion (very fair, fair, medium, dark, very dark), numbers of nevi and freckles (none, few, many, very many), and skin sensitivity to sun exposure (none, moderate, high). That questionnaire also collected data on regions of birth and of residence at baseline, which we linked to a database containing mean daily ultraviolet radiation dose in French departments obtained from the Joint Research Centre of the European Commission (27). Histories of endometriosis and uterine fibroma were available in the 1992 questionnaire and were then updated at each follow-up, along with procedures allowing detection and/or treatment for these conditions. Data on weight were available in each questionnaire, and height was collected in the questionnaires sent at baseline and in 1995, 2000, and 2002, which allowed us to compute a body mass index (calculated as weight (kg)/height $(m)^2$) at each follow-up. Data on hormonal treatments (oral contraceptives, menopausal hormone therapy, and oral progestagens taken alone before menopause) were available in each follow-up questionnaire starting from 1992.

Data reproducibility. Among participants for whom age at menarche was available in both questionnaires ($n = 79{,}282$), the kappa coefficient was 0.68, which represents substantial agreement between the 2 measures (28). The validity of menopausal status and age has previously been tested in a random sample of 151 women in our cohort by comparing self-report from the participants and medical records from their gynecologists (29). The kappa coefficient for menopausal status was 0.85. Among the 57 women that were menopausal according to both sources, the weighted kappa coefficient was 0.64 for age at menopause. Regarding menopausal hormone therapy use, the kappa coefficient was 0.74 between self-reported use in the 2005 questionnaire and data from reimbursement files concomitantly provided by medical insurance. In addition, a former validation study in our cohort yielded a correlation coefficient of 0.92 between self-reported and technician-measured body mass index (30).

Population for analysis

Participants who reported a history of cancer other than basal-cell carcinoma at baseline ($n = 4{,}788$), those who were lost to follow-up from baseline ($n = 2{,}207$), or who reported to have never menstruated ($n = 28$) were excluded, leaving us with a final sample of 91,972 women. Women-years were computed from the date of return of the first questionnaire to the date of melanoma diagnosis, date of diagnosis of any other cancer, date of death, date of last questionnaire returned, or date of end of follow-up (July 7, 2005), whichever occurred first.

Statistical analysis

Statistical analyses were performed by using the SAS, version 9.1, statistical package (SAS Institute, Inc., Cary, North Carolina). Relative risks and 95% confidence intervals were estimated by using Cox proportional hazards regression models with age as the time scale. The association between endogenous hormonal factors and melanoma risk was assessed in age-adjusted models and then in multivariate models additionally adjusted for pigmentary characteristics (hair color, skin complexion, number of nevi, number of freckles, skin sensitivity to sun exposure), mean daily ultraviolet radiation dose over spring and summer in regions of birth and of residence at baseline, and education. We further adjusted this multivariate model separately for each of the following: 1) history of endometriosis and 2) history of uterine fibroma, because we had identified positive associations between these conditions and melanoma in a previous report (31). In the latter models, we excluded endometriosis ($n = 5$) and uterine fibromas ($n = 27$) that occurred before menarche because these conditions are rare before puberty, as well as those that were not reported as treated or diagnosed through a specific procedure (endometriosis: $n = 511$; uterine fibromas: $n = 3{,}628$); 3) quartiles of body mass index (<20.5, 20.5–21.9, 22.0–23.8, ≥ 23.9 kg/m^2); 4) oral contraceptive use (ever, never); 5) menopausal hormone therapy use (ever, never) in postmenopausal women; and 6) use of progestagens taken alone before menopause (ever, never). History of endometriosis or fibroma, body mass index, use of hormonal treatments, menopausal status, and age at menopause were considered as time-dependent variables. Age at first birth or pregnancy, age at last birth, and time since last birth were categorized into quartiles. Where relevant, we performed tests for linear trend using an ordinal score for each factor. Using the same methods, we stratified our results according to menopausal status and according to median daily ultraviolet radiation dose over spring and summer in regions of birth and of residence at baseline. We then performed stratification according to tumor site and subtype using competing-risks

Table 1. Characteristics of Study Participants, E3N Cohort, 1990–2005 ($n = 92,972$)

	Incident Melanoma			
	Yes ($n = 460$)		No ($n = 92,512$)	
	No.	%	No.	%
Age at baseline, years				
<44	113	24.6	25,642	28.0
44–47	110	23.9	19,696	21.5
48–53	136	29.6	23,175	25.3
≥54	101	21.9	22,999	25.2
Educational level, years				
≤12	48	10.4	12,169	13.3
13–14	256	55.7	47,716	52.1
≥15	156	33.9	31,627	34.6
Hair color				
Blond	80	17.4	9,154	10.0
Red	24	5.2	1,520	1.7
Chestnut	280	60.9	55,208	60.3
Brown	60	13.0	21,157	23.1
Dark	16	3.5	4,473	4.9
Skin complexion				
Very fair	15	3.3	1,075	1.2
Fair	319	69.3	53,290	58.2
Medium	123	26.7	35,680	39.0
Dark/very dark	3	0.7	1,467	1.6
No. of nevi				
Very many	109	23.7	9,608	10.5
Many	225	48.9	39,723	43.4
Few	110	23.9	33,140	36.2
None	16	3.5	9,041	9.9

Table continues

models (32). Tests for homogeneity were performed to compare estimates over strata. Missing data were excluded for each endogenous hormonal factor separately, and numbers are provided as footnotes in the tables. For adjustment variables, missing values were imputed to the modal category if occurring in <5% of observations; otherwise a missing category was created. All *P* values were 2 sided.

RESULTS

A total of 460 melanoma cases were ascertained over 1,207,674 women-years. Pathology reports could be obtained for 95% of melanomas, and a total of 80.8% were invasive tumors. Characteristics of cases and noncases are described in Table 1.

Main analyses

Reproductive factors. Nulliparous women did not significantly differ from parous women with regard to melanoma risk (Table 2). There was a marginally significant decreased risk of melanoma in parous women with ≥3 births, as compared with parous women with <3 births (relative risk (RR) = 0.85, 95% confidence interval (CI): 0.68, 1.05). There was no evidence of an association between age at first birth and melanoma risk. Results for pregnancies were similar to those observed with parity. A history of miscarriages was associated with a marginally significant decreased melanoma risk (RR = 0.81, 95% CI: 0.62, 1.05). We found no evidence of an association between melanoma risk and age at last birth, time since last birth, or cumulated breastfeeding duration.

Menstrual factors. Late age at menarche (i.e., ≥15 years) was significantly associated with a decreased melanoma risk (RR = 0.67, 95% CI: 0.46, 0.97, compared with 13–14 years; $P_{trend} = 0.30$) (Table 3). While menstrual cycle length per se was not significantly associated with melanoma risk, women reporting irregular menstrual cycles were significantly at decreased risk (RR = 0.52, 95% CI: 0.31, 0.89, compared with regular cycles of 25–31 days). There was no significant association between melanoma risk and age at menstrual cycle regularity, however. Further, although menopausal status was not associated with melanoma risk, we found a marginally significant inverse association between

Table 1. Continued

	Incident Melanoma			
	Yes (n = 460)		No (n = 92,512)	
	No.	%	No.	%
No. of freckles				
Very many	44	9.6	4,662	5.1
Many	179	38.9	26,323	28.8
Few	109	23.7	22,011	24.0
None	128	27.8	38,516	42.1
Skin sensitivity to sun exposure				
High	176	38.3	25,796	28.2
Moderate	212	46.1	44,736	48.9
None	72	15.6	20,980	22.9
Mean daily UVR dose in region of birth, kJ/m^2[a]				
Quartile 1	155	33.7	26,943	29.4
Quartile 2	76	16.5	14,696	16.1
Quartile 3	98	21.3	19,403	21.2
Quartile 4	103	22.4	22,638	24.7
Missing	28	6.1	7,832	8.6
Mean daily UVR dose in region of residence at baseline, kJ/m^2[a]				
Quartile 1	136	29.6	24,091	26.3
Quartile 2	105	22.8	20,906	22.9
Quartile 3	95	20.6	19,094	20.8
Quartile 4	124	27.0	27,421	30.0
History of endometriosis[b]				
Ever	38	8.3	5,255	5.8
Never	422	91.7	85,738	94.2
History of uterine fibroma[b]				
Ever	129	29.1	21,775	24.8
Never	314	70.9	66,099	75.2

Abbreviations: E3N, Etude Epidémiologique auprès de femmes de l'Education Nationale; UVR, ultraviolet radiation.

[a] The cutoff points for quartiles of mean daily UVR dose in the regions of birth and of residence at baseline were 2.36, 2.48, and 2.69 kJ/m^2.

[b] At the end of follow-up.

risk and earlier age at natural menopause (i.e., <48 years) (RR = 0.70, 95% CI: 0.48, 1.02, compared with 48–51 years; P_{trend} = 0.13). There was also a significantly inverse relation between melanoma risk and shorter menstrual life (RR = 0.51, 95% CI: 0.28, 0.91, for <33 years compared with ≥39 years; P_{trend} = 0.30).

Results regarding reproductive and menstrual factors were generally not substantially modified after additional adjustment for history of endometriosis, history of uterine fibroma, body mass index, or use of hormonal treatments (refer to Web Table 1, the first of 3 Web tables, posted on the *Journal*'s Web site (http://aje.oupjournals.org/)). However, after additional adjustment for menopausal hormone therapy use in postmenopausal women, associations were slightly stronger between melanoma risk and age at menarche (≥15 years: RR = 0.51, 95% CI: 0.30, 0.86, compared with 13–14 years; P_{trend} = 0.53), age at natural menopause (<48 years: RR = 0.68, 95% CI: 0.46, 0.99, compared with 48–51 years; P_{trend} = 0.07), and length of ovulatory life (<33 years: RR = 0.35, 95% CI: 0.17, 0.72, compared with ≥39 years; P_{trend} = 0.04).

Stratified analyses

Stratification of analyses according to menopausal status yielded no significant heterogeneity (Table 4). The relation between parity and melanoma risk was slightly stronger in pre- than in postmenopausal women, and the association between melanoma risk and number of pregnancies was marginally significant in premenopausal women (RR = 0.67, 95% CI: 0.44, 1.00), without significant heterogeneity according to menopausal status. The relations between

Table 2. Relative Risks and 95% Confidence Intervals for Cutaneous Melanoma in Relation to Reproductive Factors, E3N Cohort, 1990–2005 ($n = 91,972$)[a]

Reproductive Factor[b]	Total No.	No. of Cases	Person-Years	Age-adjusted RR	95% CI	Adjusted RR[c]	95% CI
Nulliparous							
No	80,421	407	1,058,915	1.00	Referent	1.00	Referent
Yes	10,852	52	139,809	0.96	0.72, 1.29	0.94	0.71, 1.26
Parity[d]							
<3 births	53,598	285	704,796	1.00	Referent	1.00	Referent
≥3 births	26,783	122	353,586	0.83	0.67, 1.03	0.85	0.68, 1.05
Age at first birth, years[d]							
<22	15,227	73	199,870	1.00	Referent	1.00	Referent
22–23	17,718	85	234,477	0.99	0.72, 1.35	0.97	0.71, 1.32
24–26	24,304	142	321,158	1.20	0.91, 1.60	1.15	0.87, 1.54
≥27	22,867	106	299,740	0.97	0.72, 1.30	0.91	0.67, 1.23
P_{trend}				0.87		0.77	
Ever pregnant							
Ever	82,751	417	1,088,978	1.00	Referent	1.00	Referent
Never	8,597	42	110,709	0.98	0.72, 1.35	0.97	0.70, 1.33
No. of pregnancies[e]							
<3 pregnancies	39,443	213	517,953	1.00	Referent	1.00	Referent
≥3 pregnancies	42,236	200	556,657	0.86	0.71, 1.05	0.87	0.72, 1.05
Age at first pregnancy, years[e]							
<22	17,835	96	110,697	1.00	Referent	1.00	Referent
22–23	18,997	146	233,764	1.06	0.78, 1.42	1.04	0.77, 1.40
24–26	24,292	93	251,503	1.30	0.99, 1.71	1.26	0.95, 1.65
≥27	21,331	81	320,653	0.98	0.73, 1.32	0.94	0.69, 1.27
P_{trend}				0.71		0.99	
History of miscarriages							
No	59,186	321	789,059	1.00	Referent	1.00	Referent
Yes	14,376	65	192,155	0.83	0.64, 1.09	0.81	0.62, 1.05

Table continues

melanoma risk and age at menarche and irregularity of menstrual cycles were significant in postmenopausal women only, but estimates were based on a low number of cases in premenopausal women, and there was no evidence for heterogeneity according to menopausal status. In addition, we found no evidence for heterogeneity in estimates overall according to median daily ultraviolet radiation dose in regions of birth or of residence at baseline (data not shown).

When stratifying according to melanoma site, nulliparity was positively associated with trunk melanoma, while it was inversely associated with head and neck melanoma ($P_{homogeneity} = 0.04$), although estimates were not significant (Web Table 2). Because of small numbers in some subgroups, it would be difficult to draw conclusions regarding site-specific risk; however, the associations between melanoma risk and menstrual factors were generally similar across sites, except for age at menarche and age at natural menopause, for which risk estimates were close to unity in head and neck melanomas, and in both head and neck and trunk melanomas, respectively.

When examining estimates according to histologic type of melanoma, we found no evidence of heterogeneity (Web Table 3). Results were mostly driven by the largest subgroup (superficial spreading/nodular melanoma). Here again, although numbers were small, the associations regarding menstrual factors were comparable across subtypes, except for menstrual cycle irregularity, which was positively associated with risk of lentigo maligna/lentigo maligna melanoma, contrary to what was observed in the main analyses. Length of ovulatory life was also positively associated with melanomas of unknown/rare subtype.

DISCUSSION

The findings from this large prospective cohort suggest a significantly reduced risk of melanoma associated with later menarche, menstrual cycle irregularity, earlier age at natural menopause, and, in women in whom menopause occurred naturally, shorter length of ovulatory life. A modestly

Table 2. Continued

Reproductive Factor[b]	Total No.	No. of Cases	Person-Years	Age-adjusted RR	95% CI	Adjusted RR[c]	95% CI
Age at last birth, years[d]							
<26	16,001	82	212,701	1.00	Referent	1.00	Referent
26–28	16,885	99	226,075	1.13	0.85, 1.52	1.10	0.82, 1.48
29–31	17,005	86	228,501	0.97	0.72, 1.31	0.93	0.69, 1.27
≥32	21,456	110	284,844	1.00	0.75, 1.33	0.94	0.70, 1.26
P_{trend}				0.69		0.44	
Time since last birth, years[d]							
<28	18,755	193	215,583	1.00	Referent	1.00	Referent
28–32	15,524	88	210,630	1.11	0.82, 1.51	1.15	0.84, 1.56
33–38	18,921	91	265,973	0.92	0.61, 1.36	0.96	0.64, 1.43
≥39	18,145	35	259,947	0.77	0.43, 1.36	0.82	0.46, 1.47
P_{trend}				0.47		0.65	
Breastfeeding duration, months[d]							
Never	22,932	123	304,694	1.00	Referent	1.00	Referent
<3	19,399	102	258,695	0.99	0.76, 1.28	0.96	0.74, 1.25
≥3	28,787	150	385,778	0.96	0.76, 1.22	0.95	0.75, 1.21
P_{trend}				0.74		0.70	

Abbreviations: CI, confidence interval; E3N, Etude Epidémiologique auprès de femmes de l'Education Nationale; RR, relative risk.

[a] Tests for linear trend were performed by using an ordinal score for each factor.

[b] Totals do not add up because missing values were deleted for each factor separately: There were 699 (0.8%) missing values for nulliparity; 40 (0.1%) for parity; 369 (0.5%) for age at first birth; 624 (0.7%) for ever being pregnant; 1,072 (1.3%) for number of pregnancies; 308 (0.4%) for age at first pregnancy; 9,205 (11.1%) for history of miscarriages; 9,159 (11.4%) for age at last birth and time since last birth; and 9,303 (11.6%) for breastfeeding duration.

[c] Adjusted for age, hair color, skin complexion, number of nevi, number of freckles, skin sensitivity to sun exposure, mean ultraviolet radiation dose in regions of birth and of residence at baseline, and educational level.

[d] In parous women.

[e] In ever pregnant women.

decreased risk was also found with parity, number of pregnancies, and history of miscarriages.

Our findings regarding menarcheal age are in agreement with those from 3 case-control studies: a Danish study suggesting a decreased risk in women aged ≥16 years at menarche compared with <13 years (17); a French study in which menarche occurring between 10 and 13 years was associated with greater melanoma risk compared with menarche at ≥14 years (14); and a Canadian study reporting an 18% decrease in risk with each additional year of menarcheal age (7). In contrast, 2 case-control studies, from the United States and Australia, showed a positive relation (9, 10), while 3 others reported no evidence of an association (8, 13, 23). The association between melanoma and menstrual cycle length or regularity has rarely been explored, with no prospective data available. Thus, although a Canadian case-control study reported shorter cycles in melanoma cases compared with controls (7), a US investigation found no association between menstruation irregularity and melanoma (11). We found no significant association between menopausal status and melanoma risk, consistent with most previous reports (10, 16, 17, 23). However, the validity of these findings is questionable, given the high probability that these relations are confounded by age. Regarding age at menopause, although most studies reported no significant association with melanoma (7, 8, 12, 16, 18), one US study reported a 3.6-fold significantly increased risk of superficial spreading melanoma in women aged ≥55 years at natural menopause compared with ≤49 years (10). That finding is consistent with our results but contrasts with those from a Danish study reporting a significantly decreased risk in women with natural menopause occurring at ≥45 years compared with earlier (17). The association between length of ovulatory life and melanoma was investigated in 4 studies, which used different calculations and mostly included premenopausal women. Most found no evidence of an association (10, 15, 17), but our findings are consistent with those from an Australian study that reported a significantly positive relation (9).

Most previous studies have reported an inverse relation between parity and melanoma risk (4, 6, 8, 17, 19, 20, 22–25). Our results confirm this association, although our finding was only marginally significant. We also observed an inverse relation between melanoma risk and total number of pregnancies. Although we observed a modest inverse association between history of miscarriages and melanoma, 2 previous studies found no evidence of an association (11, 17). Regarding age at first birth, most studies yielded positive relations (4, 6, 9, 12, 16, 21, 22), while we found no evidence of an association with melanoma risk, similarly to

Table 3. Relative Risks and 95% Confidence Intervals for Cutaneous Melanoma in Relation to Menstrual Factors, E3N Cohort, 1990–2005 ($n = 91,972$)[a]

Menstrual Factor[b]	Total No.	No. of Cases	Person-Years	Age-adjusted RR	95% CI	Adjusted RR[c]	95% CI
Age at menarche, years							
<13	41,752	209	546,389	0.95	0.79, 1.15	0.95	0.79, 1.15
13–14	40,055	213	528,894	1.00	Referent	1.00	Referent
≥15	8,790	32	115,488	0.68	0.47, 0.99	0.67	0.46, 0.97
P_{trend}				0.31		0.30	
Menstrual cycle length during midlife, days[d]							
≤24	6,412	25	83,740	0.75	0.50, 1.13	0.75	0.50, 1.12
25–31	74,082	387	974,312	1.00	Referent	1.00	Referent
≥32	5,621	31	74,538	1.05	0.73, 1.52	1.01	0.70, 1.46
Irregular cycles	5,004	14	64,655	0.54	0.31, 0.91	0.52	0.31, 0.89
P_{trend}				0.21		0.27	
Age at menstrual cycle regularity, years							
<12	9,811	44	126,632	1.00	Referent	1.00	Referent
12–17	69,939	360	919,749	1.07	0.82, 1.53	1.11	0.81, 1.52
≥18	10,524	52	141,050	1.12	0.72, 1.60	1.03	0.69, 1.53
P_{trend}				0.77		0.95	
Menopausal status							
Premenopausal	5,597	106	337,331	1.00	Referent	1.00	Referent
Postmenopausal	85,143	351	861,515	1.06	0.76, 1.48	1.06	0.76, 1.48
Age at natural menopause, years[e]							
<48	10,754	36	131,508	0.69	0.47, 1.01	0.70	0.48, 1.02
48–51	33,808	148	357,758	1.00	Referent	1.00	Referent
≥52	28,119	104	252,746	1.01	0.78, 1.31	1.01	0.78, 1.31
P_{trend}				0.12		0.13	
Length of ovulatory life, years[e]							
<33	4,992	9	66,471	0.49	0.28, 0.90	0.51	0.28, 0.91
33–35	11,680	50	159,586	1.05	0.79, 1.42	1.06	0.79, 1.43
36–38	25,199	115	361,531	1.12	0.90, 1.40	1.12	0.90, 1.40
≥39	30,676	111	445,845	1.00	Referent	1.00	Referent
P_{trend}				0.26		0.30	

Abbreviations: CI, confidence interval; E3N, Etude Epidémiologique auprès de femmes de l'Education Nationale; RR, relative risk.

[a] Tests for linear trend were performed by using an ordinal score for each factor.

[b] Totals do not add up because missing values were deleted for each factor separately: There were 1,375 (1.5%) missing values for age at menarche; 853 (0.9%) for menstrual cycle length; 1,251 (1.4%) for age at menstrual cycle regularity; 1,232 (1.3%) for menopausal status; 3,148 (4.1%) for age at natural menopause; and 989 (1.3%) for length of ovulatory life.

[c] Adjusted for age, hair color, skin complexion, number of nevi, number of freckles, skin sensitivity to sun exposure, mean ultraviolet radiation dose in regions of birth and of residence at baseline, and educational level.

[d] P_{trend} excludes irregular cycles.

[e] In postmenopausal women with natural menopause.

3 other studies (8, 11, 19). We found no significant association between age at last birth and melanoma, unlike an Italian case-control study that reported a significant dose-response relation (16). We also found no evidence of an association between time since last birth and melanoma risk, which is consistent with the results from a Swedish retrospective cohort (6) but contrasts with those from a Danish prospective analysis, in which time since last birth of ≥15 years was associated with a significant 20% increase in risk compared with <10 years (21). However, time since last birth was generally elevated in our study population, which may have reduced our ability to observe such risk variation. One study explored the relation between cumulated breastfeeding duration and melanoma and showed no evidence of an association (7), consistent with our findings.

To our knowledge, no previous study has examined endogenous hormonal factors in relation to melanoma according to menopausal status. Our results should be interpreted

Table 4. Relative Risks and 95% Confidence Intervals for Cutaneous Melanoma in Relation to Endogenous Hormonal Factors Stratified According to Menopausal Status, E3N Cohort, 1990–2005 ($n = 91,972$)[a]

Endogenous Hormonal Factor[b]	Premenopausal Women				Postmenopausal Women				*P* Value[c]
	No. of Cases	Person-Years	Adjusted RR[d]	95% CI	No. of Cases	Person-Years	Adjusted RR[d]	95% CI	
Nulliparous									
No	95	300,032	1.00	Referent	310	751,478	1.00	Referent	
Yes	11	35,380	1.00	0.53, 1.87	40	103,262	0.91	0.65, 1.27	0.79
Parity[e]									
<3 births	76	215,495	1.00	Referent	207	484,179	1.00	Referent	
≥3 births	19	84,429	0.63	0.38, 1.05	103	266,883	0.92	0.72, 1.17	0.19
Age at first birth, years[e]									
<22	16	57,234	1.00	Referent	57	140,903	1.00	Referent	
22–23	18	63,754	0.96	0.53, 1.75	67	168,937	1.01	0.74, 1.37	0.89
24–26	38	89,629	1.47	0.90, 2.41	103	229,350	1.11	0.84, 1.46	0.33
≥27	23	88,543	0.92	0.52, 1.61	82	209,524	0.94	0.70, 1.26	0.95
P_{trend}			0.80				0.44		
Ever pregnant									
Ever	97	308,510	1.00	Referent	318	772,808	1.00	Referent	
Never	9	27,150	1.08	0.54, 2.14	32	82,635	0.92	0.64, 1.32	0.68
No. of pregnancies[f]									
<3 pregnancies	59	156,386	1.00	Referent	152	357,662	1.00	Referent	
≥3 pregnancies	38	148,735	0.67	0.44, 1.00	162	404,298	0.95	0.76, 1.19	0.13
Age at first pregnancy, years[f]									
<22	20	69,786	1.00	Referent	61	162,031	1.00	Referent	
22–23	20	69,188	1.02	0.55, 1.90	73	180,482	1.04	0.74, 1.47	0.95
24–26	35	88,417	1.41	0.81, 2.46	110	230,034	1.20	0.87, 1.64	0.61
≥27	22	80,316	0.99	0.54, 1.84	73	197,466	0.90	0.64, 1.28	0.79
P_{trend}			0.72				0.76		
History of miscarriages									
No	76	222,237	1.00	Referent	244	562,183	1.00	Referent	
Yes	14	54,131	0.73	0.41, 1.30	50	137,009	0.82	0.60, 1.11	0.74

Table continues

with caution, however, given the small sample size of the premenopausal subgroup, the low number of cases in extreme subgroups, and the lack of heterogeneity between pre- and postmenopausal women. Although we found differential melanoma risks in relation to nulliparity according to the anatomic site of melanoma, 2 studies reported no differential associations with parity, age at first birth, or number of pregnancies (19, 20). The associations that we observed between melanoma and menstrual factors in the main analyses were generally similar across body sites, although there were no associations between age at menarche and head and neck melanoma and between age at natural menopause and melanomas of the head and neck and of the trunk, most likely because of small numbers. Previous studies generally reported no subtype-specific associations (4, 11, 17, 20). However, a Canadian study showed a positive association between age at menarche ≥15 years compared with <13 years and nodular melanoma (8), and a US study reported a significantly higher risk of superficial spreading melanoma in women aged ≥55 years at natural menopause compared with ≤49 years, as well as a significantly increased risk of nodular melanoma in women with longer time since natural menopause (10). While our findings were generally similar across subtypes, menstrual cycle irregularity was positively associated with lentigo maligna/lentigo maligna melanoma. Although numbers were small, this association should be investigated further.

Regarding menstrual factors, our data may suggest that reduced exposure to ovarian hormones is associated with a reduced melanoma risk. These associations for melanoma are consistent with those observed for breast cancer. Women with earlier menarche and later menopause, and thus potentially higher exposure to ovarian steroids, were shown to be at increased breast cancer risk (33, 34). In addition, menstrual irregularity has been suggested to reflect anovulatory cycles and reduced exposure to sex hormones (35). These observations are consistent with earlier work showing that sex hormones have an impact on skin pigmentation (36), as is

Table 4. Continued

Endogenous Hormonal Factor[b]	Premenopausal Women				Postmenopausal Women				*P* Value[c]
	No. of Cases	Person-Years	Adjusted RR[d]	95% CI	No. of Cases	Person-Years	Adjusted RR[d]	95% CI	
Age at last birth, years[e]									
<26	21	60,317	1.00	Referent	61	150,985	1.00	Referent	
26–28	24	63,414	1.05	0.58, 1.89	73	161,309	1.09	0.77, 1.53	0.92
29–31	19	63,711	0.85	0.45, 1.58	67	163,712	0.96	0.68, 1.37	0.72
≥32	24	81,074	0.82	0.45, 1.50	86	202,180	0.98	0.70, 1.37	0.62
P_{trend}				0.40				0.71	
Breastfeeding duration, months									
Never	27	83,617	1.00	Referent	94	221,077	1.00	Referent	
<3	31	78,215	1.18	0.70, 1.98	71	180,480	0.90	0.66, 1.23	0.39
≥3	29	108,753	0.81	0.48, 1.37	121	277,024	1.02	0.77, 1.33	0.45
P_{trend}				0.40				0.88	
Age at menarche, years									
<13	51	156,993	1.05	0.70, 1.55	157	385,408	0.93	0.75, 1.15	0.60
13–14	47	147,946	1.00	Referent	165	377,532	1.00	Referent	
≥15	7	27,707	0.77	0.35, 1.71	25	86,945	0.65	0.43, 0.99	0.71
P_{trend}				0.52				0.40	
Menstrual cycle length during midlife, days[g]									
≤24	2	20,877	0.29	0.07, 1.16	22	62,277	0.83	0.54, 1.29	0.15
25–31	94	275,364	1.00	Referent	292	692,354	1.00	Referent	
≥32	6	24,719	0.69	0.30, 1.57	25	49,204	1.15	0.77, 1.74	0.27
Irregular cycles	3	13,653	0.58	0.18, 1.82	11	50,348	0.52	0.28, 0.94	0.87
P_{trend}				0.53				0.37	
Age at menstrual cycle regularity, years									
<12	6	36,248	1.00	Referent	38	89,209	1.00	Referent	
12–17	84	247,340	2.06	0.90, 4.72	274	666,052	0.95	0.68, 1.34	0.09
≥18	14	48,329	1.69	0.65, 4.40	38	92,004	0.93	0.59, 1.45	0.26
P_{trend}				0.48				0.74	

Abbreviations: CI, confidence interval; E3N, Etude Epidémiologique auprès de femmes de l'Education Nationale; RR, relative risk.

[a] Tests for linear trend were performed by using an ordinal score for each factor.

[b] Totals do not add up because missing values were deleted for each factor separately: There were 699 (0.8%) missing values for nulliparity; 40 (0.1%) for parity; 369 (0.5%) for age at first birth; 624 (0.7%) for ever being pregnant; 1,072 (1.3%) for number of pregnancies; 308 (0.4%) for age at first pregnancy; 9,205 (11.1%) for history of miscarriages; 9,159 (11.4%) for age at last birth and time since last birth; 9,303 (11.6%) for breastfeeding duration; 1,375 (1.5%) for age at menarche; 853 (0.9%) for menstrual cycle length; and 1,251 (1.4%) for age at menstrual cycle regularity.

[c] Test for homogeneity in estimates between pre- and postmenopausal women.

[d] Adjusted for age, hair color, skin complexion, number of nevi, number of freckles, skin sensitivity to sun exposure, physical activity, and mean ultraviolet radiation dose in regions of birth and of residence at baseline. Additionally adjusted for menopausal hormone therapy in postmenopausal women.

[e] In parous women.

[f] In ever pregnant women.

[g] P_{trend} excludes irregular cycles.

the observation of hormonal receptors in melanocytic lesions (37), with a higher expression of estrogen receptor β with degree of invasiveness in melanomas (38). However, although we observed a reduced risk of melanoma in the categories of menstrual factors reflecting reduced exposures to female hormones, this interpretation should be tempered by the facts that there was no clear dose-dependent trend across subgroups and that some of the associations observed relied on relatively small categories. Interestingly, our results regarding menarcheal and menopausal ages were stronger after adjustment for menopausal hormone therapy use, potentially because of an influence of menopausal hormone therapy on melanoma risk, which will be investigated further.

Regarding reproductive factors, the inverse associations between breast cancer risk and number of births and age at first birth have been suggested to be due to pregnancy

hormones conferring early and increased differentiation of the mammary gland, thus reducing the number of susceptible cells that can undergo malignancy (39). Given our results of a marginally significant inverse association between melanoma and parity, this pathway may also be applicable to melanoma. However, some part of the association could be explained by confounding through sun exposure or socioeconomic factors. It has indeed been suggested that the inverse associations that have been repeatedly reported between melanoma risk and parity and age at first birth may reflect lower sun exposure levels, with 2 particular studies showing a similar relation between number of children and melanoma risk in women and in men (21, 25).

Strengths of our study include the large sample size, the prospective design with collection of endogenous hormonal factors at inclusion (thus prior to melanoma diagnosis), and the long duration of follow-up. Except for number of births, our study regarding other endogenous hormonal factors is to date the one that includes the largest number of melanoma cases within a prospective design. We had access to detailed data on reproductive and menstrual factors and were able to stratify analyses according to menopausal status. Almost all melanoma cases were ascertained through pathology reports, which allowed us to perform stratified analyses according to melanoma site and subtype. Moreover, findings were adjusted for the main established melanoma risk factors (i.e., pigmentary characteristics, mean daily ultraviolet radiation dose in the region of birth and in the region of residence at baseline, education), although sun exposure patterns were not available. However, although the role of sun exposure on melanoma risk has been well established in ecologic studies (40, 41), analytical epidemiologic studies typically fail to show robust associations; therefore, we speculate that adjustment for this factor would have had little effect on our findings. In addition, our results were quite stable after adjustment for ambient ultraviolet radiation dose in the regions of birth and at baseline. Endogenous hormonal factors were self-reported by the participants, which may have induced some level of recall bias and misclassification. However, we found a high level of reproducibility in age at menarche, menopausal status, and age at menopause; also, because systematic errors in the report of endogenous hormonal factors are unlikely to be differential between cases and noncases, overestimation of effects is unlikely to have occurred. Finally, no correction was made for multiple testing and, given the multiple tests performed, we cannot exclude the possibility that some of our results may have occurred by chance.

In conclusion, our findings from this large prospective cohort study may suggest that melanoma risk is reduced with decreased exposure to ovarian hormones. However, no clear dose-dependent trend was observed in our data, and additional epidemiologic studies are needed to prospectively examine these relations while including sun exposure measures collected prior to melanoma diagnosis, in order to adequately take these factors into account. Experimental studies should further explore the influence of sex hormones on melanoma and other melanocytic lesions to help improve our understanding of this complex disease.

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