Short Communication

Serum Follicle-Stimulating Hormone and Risk of Epithelial Ovarian Cancer in Postmenopausal Women

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Abstract

The "gonadotropin hypothesis" postulates that gonadotropin overstimulation of ovarian epithelium results in its increased proliferation and subsequent malignant transformation. To address this hypothesis, we assessed the association between prediagnostic serum levels of follicle-stimulating hormone (FSH) and the risk of epithelial ovarian cancer in postmenopausal women who were part of a case–control study nested within three prospective cohorts in New York City, Umeå, Sweden, and Milan, Italy. Case subjects were 88 women with primary invasive epithelial ovarian cancer diagnosed between 3 months and 13.1 years after the blood donation. Controls were 168 women who were free of cancer and matched the case on cohort, age, and enrollment date. Serum FSH was determined using a quantitative immunoradiometric assay. FSH concentrations were similar in women who subsequently received a diagnosis of epithelial ovarian cancer (median, 44.0 mIU/ml; range, 13.8–101.2) and in controls (median, 43.4 mIU/ml; range, 13.5–109.5; $P = 0.17$). Compared with women in the lowest third, women in the highest third of serum FSH were not at increased risk of epithelial ovarian cancer after an adjustment for potential confounders (odds ratio, 0.85; 95% confidence interval, 0.36–1.99). These observations provide no evidence for an association between circulating FSH and risk of epithelial ovarian cancer in postmenopausal women and do not appear to support the gonadotropin hypothesis of epithelial ovarian carcinogenesis.

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Introduction

Ovarian cancer is the second most common cancer of the female reproductive system and the leading cause of death from gynecological neoplasms in the United States (1). The American Cancer Society estimated that 25,400 women will be diagnosed with ovarian cancer, and 14,300 will die from the disease in 2003 (1). Although hereditary ovarian cancer is thought to account for 5–10% of all cases (2), the etiology of ovarian cancer in the majority of cases remains poorly understood (3). Endogenous hormones are thought to play an important role in ovarian carcinogenesis but the direct evidence is limited (4, 5). On the basis of experimental data suggesting that high gonadotropin [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] secretion is associated with ovarian tumors in animal models, Cramer and Welch (6) proposed the gonadotropin hypothesis of ovarian cancer pathogenesis. The hypothesis states that excessive gonadotropin and estrogen stimulation of the ovarian inclusion cysts (formed through repeated invaginations of ovarian epithelium during incessant ovulations) results in increased proliferation and malignant transformation of ovarian epithelium. In contrast, pregnancy and the use of oral contraceptives protect against ovarian cancer by lowering gonadotropin levels.

Although the gonadotropin hypothesis remains one of the leading theories of ovarian carcinogenesis to date, only two studies, both based on prospective cohorts, have directly examined the role of pituitary gonadotropins in ovarian cancer. In disagreement with the hypothesis, Helzlsouer *et al.* (7) reported an inverse association of both serum LH and serum FSH with ovarian cancer. In a previous study from our group, we observed no association between circulating LH and the risk of epithelial ovarian cancer (8).

In the present study, we examined the association between circulating FSH and the risk of epithelial ovarian cancer in a nested case–control study of postmenopausal women using samples collected in advance of clinical diagnosis from three collaborating prospective cohorts.

Materials and Methods

Study Population. The prospective cohorts in this study included the New York University Women's Health Study (NYUWHS), the Northern Sweden Health and Disease Study (NSHDS), and the Italian study of Hormones and Diet in the Etiology of Breast Tumors (ORDET). The cohorts have been described in detail previously (9–11).

Reproductive and medical history and demographic and lifestyle information were collected at enrollment and/or using follow-up questionnaires. Subjects who reported hormone use

at baseline were not eligible in the NYUWHS and the ORDET cohorts, and potential case and control subjects from the NSHDS who reported use of exogenous hormones were also considered ineligible for this study. At recruitment, all of the cohort members were asked to donate venous blood samples, which have been stored at −80°C for subsequent biochemical analyses. Samples chosen for this study were selected among the specimens that had not been thawed previously.

Nested Case–Control Study. Eligible cases were postmenopausal subjects with primary, invasive epithelial ovarian cancer diagnosed at least 2 months after the initial blood donation and identified within each parent cohort by the date of the last complete follow-up. The restriction of study to postmenopausal women was based on the results of a preliminary reliability study of the NYUWHS subjects with repeated serum FSH measurements, which showed high intraclass correlation coefficient [0.70; 95% confidence interval (CI), 0.55–0.82] for postmenopausal women and low intraclass correlation coefficient (0.09; 95% CI, 0.0–0.54) for premenopausal women over a 1-year period (12).

In the NYUWHS, follow-up consists of periodic contacts with subjects by mail and telephone, as well as record linkages with the statewide tumor registries of New York, New Jersey, Connecticut, and Florida, and with the United States National Death Index. In the NSHDS, ovarian cancer cases were identified through linkages with regional and national cancer registries, and the vital status of the study participants was ascertained by record linkage to the regional and national registries for all-cause mortality. The ORDET database was linked to the regional Lombardy Cancer Registry to identify ovarian cancer cases, and to the regional residents' files to verify the vital status of the cohort members. The dates of last complete follow-up were January 1998, December 2000, and January 1997 for the NYUWHS, NSHDS, and ORDET, respectively. A total of 88 postmenopausal epithelial ovarian cancer cases met the eligibility criteria and were included in this study from the three parent cohorts (50 cases from the NYUWHS, 29 cases from the NSHDS, and 9 cases from the ORDET study).

For each case, two controls per case were randomly selected among appropriate risk sets. The risk set for a given case included cohort subjects who were alive and free of cancer, had not had a bilateral oophorectomy, and matched the case on cohort, age (±6 months), date of recruitment (±3 months), and menopausal status at enrollment. At the time of enrollment, women were classified as postmenopausal if they reported absence of menstrual cycles in the previous 6 months, a total bilateral oophorectomy, or, if their age was 52 years or older, a hysterectomy without total oophorectomy. The success of matching for menopausal status was confirmed by FSH measurements, as described previously (13, 14). A total of 176 eligible matching controls were identified. Eight potential controls were excluded because of the lack of serum. As a result, a total of 88 epithelial ovarian cancer cases and 168 matching controls were included in the study.

The Institutional Review Boards of the New York University School of Medicine, the University of Umeå, and the Istituto Nazionale Tumori in Milan reviewed and approved the present study.

Laboratory Methods. Biochemical analyses of serum samples were performed at the hormonal laboratory of the IARC (Lyon, France). Laboratory personnel were blinded in regard to subjects' case–control status. Samples of subjects from the same match sets were always analyzed in the same batch. Serum FSH was measured by an immunoradiometric assay (IRMA; Diagnostic System Laboratories, Webster, TX). The FSH immunoradiometric assay is a noncompetitive assay in which the analyte is sandwiched between two antibodies. The first antibody is coated on the walls of the tubes used in the analysis, and the second antibody is radiolabeled for detection. The unbound fraction is removed by a washing step. The amount of radioactivity counted in the assay tubes is directly proportional to the amount of analyte in the sample. A set of standards with known amount of FSH is used to plot a standard curve from which the amount of FSH in the samples can be calculated. Assay sensitivity was as follows: all reported sample values were above the 0.11-mIU/ml lower-detection limit. As to assay specificity, the FSH kit manufacturer reported no measurable cross-reaction with other gonadotropins (LH and human chorionic gonadotropin). The within-assay coefficients of variation provided by the laboratory were ranging from 3.2 to 4.6% depending on the serum FSH concentration.

The FSH cutoff point of 13.5 mIU/ml was selected based on data provided by the kit manufacturer and an analysis of the distribution of FSH levels according to menopausal status as assessed by questionnaire, as well as according to age (<42 and >55 years), using data from more than 300 women from the three cohorts.

Statistical Analyses. To reduce departures from the normal distribution, we log-transformed continuous variables, such as FSH levels, weight, height, and body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters). A mixed-effects linear regression model was used to compare hormone levels and other continuous variables between cases and controls taking into account the matched design (15). Categorical characteristics of cases and controls were compared using the conditional logistic regression model.

To compute ORs for ovarian cancer, FSH measurements were categorized into tertiles, using the frequency distribution of cases and controls combined. The data were analyzed using conditional logistic regression. ORs were computed relative to the lowest tertile. The effect of potential confounding variables (age at menarche, parity, use of oral contraceptives, and BMI) was assessed by including these variables one at a time in the logistic regression model containing FSH, as well as by including them simultaneously. When assessing the effect of BMI, height was also included in the model as recommended by Michels *et al.* (16). Likelihood ratio tests were used to assess statistical significance and *P*s for trends. All reported *P*s are two-tailed, and *P*s <0.05 are considered statistically significant. The statistical analyses were performed using SAS Version 8.0 (SAS Institute Inc., Cary, NC).

Results

The majority of study subjects self-reported ethnicity as Caucasian (82%) and the rest as African American (3%), Hispanic (1%), and mixed/unknown (14%). The ethnic composition of the study base reflects the characteristics of the population in the participating cohorts. Selected characteristics of the cases and the controls are presented in Table 1. Because of the matching, ovarian cancer cases and controls had similar age at enrollment. There were no significant differences in age at menarche or age at first full-term pregnancy between the cases and controls. As compared with controls, ovarian cancer cases were more likely to report nulliparity (27 *versus* 17%, $P = 0.08$), later age at menopause (mean, 50.2 years *versus* 48.8 years; $P = 0.06$), and a positive first-degree history of breast cancer (17 *versus* 15%, $P = 0.57$), whereas they were less likely to report use of oral contraceptives (14 *versus* 21%, $P =$

Table 1 Characteristics of study subjects

Characteristic	Cases ($n = 88$)	Controls ($n = 168$)	P^a
Age at enrollment,[b] yr, mean (SD)	58.7 (5.1)	58.8 (5.0)	
Age at menarche, yr, mean (SD)	12.8 (1.6)	12.9 (1.5)	0.60
Age at first full-term pregnancy, yr, mean (SD)	24.9 (6.0)	24.8 (4.9)	0.56
Age at menopause, yr, mean (SD)	50.2 (4.4)	48.8 (5.5)	0.06
First-degree family history of breast cancer, *n* (%)			
No	73 (83%)	141 (85%)	
Yes	15 (17%)	25 (15%)	0.57
Parity, *n* (%)			
Never pregnant	23 (27%)	29 (17%)	
Ever pregnant	63 (73%)	138 (83%)	0.08
Oral contraceptive use, *n* (%)			
Never	62 (70%)	106 (63%)	
Ever	12 (14%)	36 (21%)	0.07
Unknown	14 (16%)	26 (16%)	
Hormone replacement therapy, *n* (%)			
Never	48 (55%)	106 (63%)	
Ever	26 (29%)	42 (25%)	0.34
Unknown	14 (16%)	20 (12%)	
Cigarette smoking, *n* (%)			
Never	44 (50%)	69 (41%)	
Ever	28 (32%)	65 (39%)	0.08
Unknown	16 (18%)	34 (20%)	
Height (cm), mean (SD)	161.0 (7.0)	161.9 (5.7)	0.32
Weight (kg), mean (SD)	66.0 (11.0)	69.9 (13.1)	0.03
Body mass index (kg/m^2) mean (SD)	25.4 (3.9)	26.6 (4.5)	0.05

[a] Mixed effect linear regression model for continuous variables (after natural log-transformation); conditional logistic regression for categorical variables.
[b] Matching criteria.

0.07; Table 1). Statistically significant differences were observed for weight and BMI. In agreement with a previous report based on the same cohorts (14), ovarian cancer cases had lower mean weight than did controls (66.0 *versus* 69.9 kg, $P = 0.03$) and lower BMI (25.4 *versus* 26.6 kg/m^2, $P = 0.05$).

The median age at diagnosis of ovarian cancer was 64 years in the NYUWHS, 60 years in the NSHDS cohort, and 64 years in the ORDET cohort. For all of the cases combined, the median period from the initial visit to the diagnosis of ovarian cancer was 4.8 years (range, 3 months to 13.1 years). Common epithelial-type ovarian tumors were observed, such as serous ($n = 38$), mucinous ($n = 10$), endometrioid ($n = 9$), clear cell ($n = 4$), mixed ($n = 1$), and not-otherwise-specified ($n = 26$) adenocarcinoma.

Table 2 reports the means, medians, SDs, and ranges of serum FSH levels in ovarian cancer cases and controls. Among all of the study subjects, the median levels of FSH were 4.5% higher among cases (44.0 mIU/ml) than among controls (42.1 mIU/ml; $P = 0.17$). No statistically significant differences in serum FSH were observed between cases and controls in the total study population, between or within the participating cohorts (Table 2).

The associations between serum FSH levels expressed as tertiles determined for all cohorts combined and the risk of epithelial ovarian cancer are reported in Table 3. No statistically significant trends were observed in unadjusted analyses (OR in the top tertile, 1.19; 95% CI, 0.63–2.24; $P_{trend} = 0.59$). Similarly, no significant associations between serum FSH and risk of ovarian cancer were observed in analyses adjusting for potential confounders (age at menarche, parity, oral contraceptives use, BMI, and height) included simultaneously in the statistical model (OR in the top tertile, 0.85; 95% CI, 0.36–1.99; $P_{trend} = 0.71$). Among the potential confounders, adjustment for BMI and height had the most effect on risk estimate (OR, 0.84; 95% CI, 0.41–1.71) for the top FSH tertile compared with the lowest FSH tertile. Models that were both unadjusted and adjusted simultaneously for potential confounders are presented (Table 3). Excluding subjects with FSH values below 30 mIU/ml (a more conservative cutoff point to assess menopausal status) did not alter the observed associations.

To determine whether the presence of early or occult disease at the time of blood donation may affect the serum levels of FSH, we examined the FSH levels stratified by lag time between blood donation and date of diagnosis (<3 years, 3–6 years, >6 years). After stratification, neither significant trends nor significant differences in serum FSH were observed between ovarian cancer cases and controls. After the exclusion of subjects with diagnoses within 3 years after blood donation, adjusted OR for the top tertile was 0.84 (95% CI, 0.29–2.44; $P_{trend} = 0.78$).

Discussion

In a case–control study nested within three prospective cohorts composed mostly of Caucasian women, circulating FSH did not appear to be associated with an increased risk of postmeno-

Table 2 Mean, SD, median, and range of serum follicle-stimulating hormone (FSH) levels among postmenopausal epithelial ovarian cancer cases and controls

Serum FSH, mIU/ml	Cases	Controls	P^a
NYUWHS			
n	50	96	
Mean (SD)	47.0 (19.1)	44.3 (14.1)	0.57
Median (range)	42.8 (18.3–101.2)	45.4 (13.5–86.9)	
NSHDS			
n	29	55	
Mean (SD)	48.1 (15.6)	42.6 (17.0)	0.13
Median (range)	47.6 (17.4–92.0)	41.2 (17.3–109.5)	
ORDET			
n	9	17	
Mean (SD)	44.0 (19.9)	40.7 (13.5)	0.92
Median (range)	43.7 (13.8–78.5)	41.1 (19.7–82.8)	
Total			
n	88	168	
Mean (SD)	47.1 (18.0)	43.4 (15.0)	0.17
Median (range)	44.0 (13.8–101.2)	42.1 (13.5–109.5)	

[a] Mixed-effect linear regression model (after natural log-transformation).

Table 3 Odds ratios (ORs) of ovarian cancer for tertiles of serum follicle-stimulating hormone (FSH) levels in postmenopausal women

Tertiles FSH, mIU/ml	Cases ($n = 88$)	Controls ($n = 168$)	OR (95% CI)[a]	
			Unadjusted[b]	Adjusted[c]
<38.0	28 (32%)	57 (34%)	1.00	1.00
38.0–49.0	28 (32%)	57 (34%)	0.99 (0.52–1.89)	0.89 (0.39–2.02)
>49.0	32 (36%)	54 (32%)	1.19 (0.63–2.24)	0.85 (0.36–1.99)
P_{trend}			0.59	0.71

[a] CI, confidence interval.
[b] Analysis matched on age and date of blood donation.
[c] Adjusted for age at menarche (continuous), parity (never pregnant, ever pregnant), oral contraceptives use (never, ever), body mass index (continuous, after natural log-transformation), and height (continuous, after natural log-transformation).

pausal epithelial ovarian cancer. This finding, along with our previous study showing a lack of association with circulating LH (8), is not consistent with what would be predicted on the basis of the gonadotropin hypothesis of ovarian carcinogenesis.

Among the strengths of the study, the major one is that serum samples were collected long before the clinical diagnosis of cancer, thus greatly reducing the possibility of an effect of the disease on circulating FSH. Also, the potential for selection bias was minimized because the cases and the controls were, by definition, from the same source population, thus ensuring ideal comparability between them. The prospective design of the study also reduced the potential for survivor bias, which is important for diseases characterized by poor prognosis such as ovarian cancer.

The study had also some limitations, the most notable one being that even after combining the resources of three large prospective cohorts, only 88 ovarian cancer cases were available for study. Another limitation is that the study had to be restricted to postmenopausal women as a result of a previous reliability assessment of serum FSH that was conducted within the NYUWHS cohort. The reliability study showed acceptably modest variability in repeat samples from the same subject in postmenopausal women (intraclass correlation coefficient, 0.70), but not in premenopausal women (intraclass correlation coefficient, 0.09; Ref. 12). Given such large intrasubject variability, it would have been impossible to examine reliably the association of interest in the premenopausal group. The role of FSH in ovarian carcinogenesis could be especially relevant for younger premenopausal women characterized by substantially more elevated frequency and amplitude of FSH pulsatile secretion than older women (17, 18).

In the only previous study that examined directly the role of gonadotropins in ovarian cancer, Helzlsouer *et al.* (7) examined LH, FSH, and other hormones in a population-based cohort in Washington County, Maryland. Of 20,305 participants who were followed up for more than 15 years after providing blood samples, 31 developed ovarian cancer. The cases were matched to 62 control subjects on age, menopausal status, and, for premenopausal women, number of days from the beginning of the last menstrual period. Contrary to the expectations, mean levels of FSH were lower among the ovarian cancer cases as compared with the controls (43.3 *versus* 54.4 IU/L, respectively; $P = 0.04$) and the risk of ovarian cancer was inversely associated with both LH and FSH (7).

Similar to the study by Helzlsouer *et al.*, we have found no evidence of positive association between serum gonadotropins and epithelial ovarian cancer. Unlike the study by Helzlsouer *et al.*, which reported a statistically significant reduction of ovarian cancer risk in women at the highest tertile of serum FSH (OR, 0.1; 95% CI, 0.0–1.0; $P_{trend} = 0.02$), our study does not corroborate such a protective association, at least in postmenopausal women.

The gonadotropin hypothesis postulates that critical events in ovarian cancer pathogenesis are the entrapment of surface epithelium in inclusion cysts followed by stimulation of the entrapped epithelium by estrogens in the presence of high and persistent levels of circulating gonadotropins (6). The original rationale for the hypothesis was based on observations suggesting that elevated gonadotropin production induced ovarian tumors in rats whose ovaries were autotransplanted to the spleen (19, 20). However, in these experimental models, tumors were exclusively of stromal origin (luteomas and granulosa cell tumors) and do not reflect the tumors of epithelial origin most commonly observed in humans.

Additional observations appear to be in conflict with the gonadotropin hypothesis. For example, the hypothesis postulates that elevated gonadotropins observed in premature ovarian failure or in early menopause should be associated with increased ovarian cancer risk, but there is no evidence to suggest that early menopause is related to ovarian cancer (21–23). Furthermore, estrogen replacement therapy, which reduces circulating gonadotropins (24) and, thus, should confer protection, appears to be associated with a moderately increased risk of ovarian cancer (25, 26). Finally, one of the mechanisms leading to excessive gonadotropin production is exposure to medications affecting the normal inhibitory feedback between ovary and pituitary, including psychotropic drugs, barbiturates, antihistamines, and anti-inflammatory drugs. However, there is no consistent evidence indicating that the use of such medications increases the risk of ovarian cancer. On the contrary, there is growing evidence that nonsteroidal anti-inflammatory drugs may actually decrease ovarian cancer risk (27–30).

Several new hypotheses of ovarian carcinogenesis were proposed recently. Ghahremani *et al.* (31) suggested that the dysregulation of apoptosis, specifically the failure to eliminate ovarian surface epithelial cells entrapped in the inclusion cysts, may play a role in ovarian tumor formation. Experimental data from primates indicate that progestin-containing oral contraceptives induce apoptosis in the ovarian epithelium (32, 33). Because combined oral contraceptives have been shown consistently to confer protection against epithelial ovarian cancer (34, 35), the induction of apoptosis could be one of the mechanisms responsible for this effect (32).

More recently, Ness *et al.* (36, 37) proposed that chronic inflammation may play a critical role in the pathogenesis of epithelial ovarian cancer. Supporting such a role are the findings that factors enhancing local inflammation (ovarian endometriosis, pelvic inflammatory disease, talc and/or asbestos exposure) are associated with increased risk of ovarian cancer (36). On the other hand, factors reducing local inflammation, such as hysterectomy without oophorectomy, tubal ligation, and the use of anti-inflammatory medications, tend to have a protective effect (36). The inflammation hypothesis may also explain the protective effect observed with reduced lifetime number of ovulations (38, 39). Mammalian ovulation has many characteristics of an inflammatory reaction, including local elevation of pro-inflammatory cytokines, prostaglandins, and leukotrienes (40, 41). Consequently, decreased number of ovulations (due to pregnancy, lactation, or oral contraceptive use) may result in less exposure of ovarian epithelium to pro-inflammatory cytokines, which are thought to play a critical role in the pathogenesis of ovarian cancer (42, 43).

In conclusion, in a prospective study of postmenopausal women, there was no evidence of a strong association between circulating FSH and risk of epithelial ovarian cancer, although larger studies are needed to evaluate more moderate (less than 2-fold) associations. The present study results do not appear to support the gonadotropin hypothesis of ovarian carcinogenesis. New theories should be explored, including novel hypotheses on the role of apoptosis and chronic inflammation in epithelial ovarian cancer.

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