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14. ABSTRACT We propose a training grant to recruit and train two postdoctoral students and three physicians, but have been able to extend that number to four postdoctoral students and four physician trainees. These trainees will acquire skills in the epidemiology and prevention of breast cancer. They will work closely with mentors who have a long track record of training Epidemiologists. The funding will allow our research group to focus specific training opportunities on breast cancer. The on-going epidemiologic studies and prevention trials offer a unique resource in which trainees can participate in cutting edge research and acquire skills that will establish them as future leaders. As of the end date of this award, we have successfully jettisoned the careers of four doctoral students and four physicians. As a result of this award the seminar established to explore specialized topics in depth is permanent and will be offered every spring through the Epidemiology department at Harvard School of Public Health.					
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Introduction

We have proposed a training grant to recruit and train two doctoral students and three physicians. In analyzing the budget going into the final year, we requested an extension of one year to finish spending on the grant. We anticipated this carryover and recruited two additional doctoral students and an additional physician MPH candidate. These trainees acquired skills in the epidemiology and prevention of breast cancer. They worked closely with experienced mentors. This funding has helped our research group focus specific training opportunities on breast cancer. The on-going epidemiologic studies and prevention trials offered unique resources with which trainees participated in cutting edge research. At the conclusion of this program, all trainees have acquired skills that will establish them as future leaders

BODY

(Approved Statement of Work is italicized)

*We will advertise and recruit **one pre-doctoral candidate** for the first year of this proposed training program.* We did not recruit in the first year (year one was expected to begin 7/1/00) due to funding not being received until September 2000 we were delayed in starting the recruitment process.

*We will advertise and recruit **one physician for a two-year training** opportunity that includes course work in the first year and research on one of the ongoing studies in the second year.* We recruited Dr. Ann Partridge, MD whose research focuses on the assessment, perception and communication of breast cancer risk as well as other aspects of provider-patient communication in oncology. She earned her M.P.H. while being supported by this fellowship.

*We will recruit **a second pre-doctoral candidate** to begin training in the second year.* We have recruited two pre-doctoral students, Heather Baer and Heather Eliassen, to make up for the first year. **Ms. Baer** has received her doctorate from HSPH. She did publish her findings on adolescent diet and benign breast disease and has made further progress on her research in the field of breast cancer etiology and prevention. Her thesis research focused on identifying factors in early life, childhood, and adolescence that influence subsequent risk of breast cancer and benign breast disease. She is using data from the Nurses' Health Study and the Nurses' Health Study II to conduct this research. She presented the findings from her first thesis project, "Body fatness at young ages and incidence of premenopausal breast cancer," at the Nurses' Health Study External Advisory Board Meeting and at the annual meeting of the Society for Epidemiologic Research. She completed this manuscript submitted it to the Journal of the National Cancer Institute. She has written another manuscript, "Early life factors and incidence of benign breast disease," and has submitted this for publication. Finally, she has begun the analysis for a third manuscript, "Childhood socioeconomic status in relation to age at menarche and breast cancer risk," and will be writing the manuscript. In addition to her thesis research, she has worked with Dr. Graham Colditz, on several other projects. In particular, she has assisted with the collection and analysis of data and preparation of manuscripts for a nested case-control study of benign breast lesions as markers of breast cancer risk, which involves collaboration with several breast pathologists at Beth Israel-Deaconess Medical Center. She has also worked on the preparation of a chapter about breast cancer for a cancer epidemiology textbook, the data analysis and preparation of a manuscript on the assessment of diet among low-income Native American and Caucasian pregnant women, the preparation of a renewal for a grant to examine predictors of benign breast disease and risk factors for breast cancer among women with benign breast disease, and the preparation of a short grant proposal to examine the relationship between childhood adiposity and concentrations of sex hormones in girls.

Ms. Eliassen's dissertation which addressed potential lifestyle factors that may lower risk of breast cancer. She received her doctorate, also while a trainee. The first paper addressed the association between use of the statin family of lipid-lowering drugs and breast cancer. She submitted an abstract and presented my work on this topic at a symposium titled Breast Cancer Research at Harvard. She completed the manuscript, and has submitted it for publication. Her second paper addresses the relation of tubal sterilization with incidence of breast cancer. She submitted an abstract on this topic to the annual Society for Epidemiologic Research meeting" She traveled to Salt Lake City for the annual meeting in June 2004 to present a poster on her analysis thus far. She has also submitted this for publication. Another topic addresses the association between weight gain and loss in adult life and incidence of breast cancer. I have begun analyses on this last topic, and plan to write up a manuscript this fall. She has also worked with her advisor, Dr. Susan Hankinson, on analyses on sex steroid hormones, prolactin II, and breast cancer incidence. She will be second author on two papers published from this work.

In the last year of the grant and after a budget analysis resulting in an extension, we recruited a

third Jeanne Marie Gaare-Eby and a fourth to take advantage of this fellowship, Sonia Mathews. **Ms. Gaare-Eby** has been taking courses for doctoral program in epidemiology at HSPH. She has also been involved in research on benign breast disease, specifically looking at the relationship between fibroadenomas and papillomas and the risk of benign breast disease in the Nurses' Health Study II cohort. As of this final report, Ms. Eby has completed two years of course work, which included epidemiologic methods, biostatistics, cancer epidemiology, biology, and prevention, in working towards her epidemiology doctorate and has passed the written qualifying examination. Currently, she has two projects underway: A nested case-control study in the Nurses' Health Study cohort investigating the relationship between C-reactive protein and breast cancer. The second is a prospective cohort analysis of the association between aspirin/NSAIDS use and hip fractures using the NHS data.

Sonia (Mathews) Maruti's major research interests involve studying the effects of diet, physical activity, and obesity - as they occur in adolescence and adulthood - and their subsequent impact on breast cancer. She is also interested in applying knowledge generated from research toward public health prevention strategies. The following is activities related to this research that she has completed: She has completed data collection for three groups of participants: the members of NHSII, their mothers and their offspring; Presented results of analysis at the "Breast Cancer Research at Harvard Symposium"; Wrote abstract for the "Breast Cancer Research at Harvard Symposium", which was accepted; Submitted manuscript to the American Journal of Epidemiology¹³ (She is currently in the process of re-submitting a revised manuscript, responding to feedback from the editor). As of this last report, Sonia has completed her third year in the epidemiology doctoral program as has been working on her thesis papers. She has almost completed a study evaluating a food frequency questionnaire (HS-FFQ) asking adults about their high school diet and it is in manuscript form. She has also begun two new projects examining the relationship between physical activity and breast cancer in the NHS II cohort. The first will involve analyzing the prospective relationship of physical activity at specific ages and throughout the lifetime. The second study is methodological, designed to assess the degree in which recall and selection bias can account for the inconsistent results of earlier research examining physical activity and breast cancer. She is currently working to collect data for this nested case-control study (n~2000), before analysis can begin.

*During the second year we will advertise for **two physicians** to begin training in the third year.* We successfully recruited Dr. Anne Partridge, Dr. Larissa Nekhlyudov and Dr. Candice Aitken and Dr. Erica Meyer who's research interests involve working with the breast oncology center at Dana Farber Cancer Institute where she plans to focus on treatment of breast cancer in the elderly.

Dr. Larissa Nekhlyudov, this year continued to work in the area of women's decision making in breast cancer prevention and early detection. The DOD fellowship specifically supported my work on studying the health related quality of life (HRQoL) among women with ductal carcinoma in situ (DCIS) of the breast. This study investigates prospectively using the Nurses' Health Study population, the HRQoL in women diagnosed with DCIS. Our early results suggest that the DCIS diagnosis does not have detrimental effects on women's overall HRQoL; however, there may be some short-term declines. She presented these results at the national meeting of the Society of General Internal Medicine held in Chicago in May 2004¹. Her oral presentation was nominated for best presentation by junior faculty. Additional analyses are currently underway and a manuscript will be submitted by October 2004. The fellowship also helped support her effort in developing and submitting an R01-equivalent research proposal to the American Cancer Society in October 2003 (resubmitted in April 2004). The goal of the proposal is to develop an effective means of communicating information to women about breast cancer screening. In addition to the above projects, She is the lead investigator on two studies, one addressing screening mammography among women in their 40's, the other is aiming to improve the management of breast symptoms by primary care providers. She is also a co-investigator on two NCI-funded studies determining the predictors of DCIS recurrence and patient-oriented outcomes of prophylactic mastectomy.

The other physician recruited to begin last year is **Dr. Candice Aitken**, received her Masters in Public Health, Clinical Effectiveness Pathway. Working with Dr. Graham Colditz, she sought to use the Nurses Health Study database to discover possible risk factors for the development of estrogen receptor negative breast cancers. In collaboration with Marco Romani and Delin Shen, they ran exploratory analysis to search for a correlation between data collected in the NHS and the development of an ER negative breast cancer.

No conclusive risk factors were determined as of yet. This work is still ongoing. She also sought to develop an algorithm for use in the primary care setting to help a woman determine her particular risk of breast cancer using known personal parameters compared against the Colditz and Rosner cumulative risk of breast cancer to age 70 model. Again with Marco Romani and Delin Shen, they sought to use Bayesian analysis to predict the most important predictors of breast cancer risk. They identified the following four inputs: oral contraceptive use duration, family history, BMI slope and history of benign breast disease. They are in the process of refining the analysis and preparing an abstract for submission to ASCO (Dec 2004) and also a manuscript for publication. Working with Dr. Harvey Mamon, she designed and wrote a protocol that seeks to use stereotactic body radiotherapy in the treatment of liver metastases. She attended the 2nd and 3rd annual SBRT meetings to learn about the work being performed by other groups in this area of research. The protocol had been approved by the IRB at the Harvard Cancer Center and has been activated. She will be attending a training session in Wurzburg, Germany to use the stereotactic body frame and treat patients with this highly conformal technique. In addition, she is working on a new protocol to use this technology in the treatment of stage I medically inoperable lung cancer. Together with Dr. Harvey Mamon, they wrote a review article for publication in Hematology and Oncology summarizing the current literature on sphincter-sparing therapy for rectal cancer. This was published in Dec 2003. Working with Dr. Anthony D'Amico, she evaluated endorectal MRI in a PSA-screened population. This work was submitted in abstract form to ASTRA and was accepted for a poster presentation. This work was the focus of my resident seminar. The preparation of the manuscript is in progress and will be submitted for publication in the coming weeks.

In the past year we also supported **Dr. Erica Mayer** while she completed her M.P.H. degree at HSPH. She has several current research projects going on, all of which have been assisted by the grant. Dr. Mayer is primarily focusing on development of emerging therapies in the treatment of breast cancer, with her most recent work in the area of anti-angiogenesis agents. She is also looking at survivorship issues in breast cancer patients. Her current protocols are:

1. DFCI protocol 04-259: A Phase I Trial of Capecitabine and ZD1893 (IRESSA) Combination Therapy in Women with Advanced Breast Cancer
2. DFCI protocol 05-160: A Phase II Study of AZD2171 in Breast Cancer Stage IV
3. DFCI protocol 05-055: Anti-Angiogenesis Treatment after Pre-operative Chemotherapy: A Pilot Study in Women with Operative Breast Cancer
4. DFCI protocol 05-129: Survivor Care after Cancer Survey

The first study, 04-259, is almost completed and will be presented in poster form at the upcoming Breast Cancer: Current Controversies and New Horizons in Boston this July. 05-160 is conditionally approved by the IRB and hopefully will open in the next 2 months; 05-055 just opened. She developed 04-259 with the assistance of Eric Winer; the next two with the assistance of Hal Burstein. 05-129 is a questionnaire study which I am working on with Craig Earle and is currently in pilot stages.

During the first year we will develop and implement an advanced seminar in breast cancer. This will bring new depth to course work not previously available at the Harvard School of Public Health. This seminar will cover topics in detail and will span from basic biology of the breast, to early

lesions, epidemiologic risk factors, statistical models of breast cancer incidence and issues in risk stratification and counseling for prevention. Going into the second year of the grant, an eight-week seminar was developed and implemented specifically for breast cancer epidemiology, covering such topics as modeling breast cancer risk, postmenopausal hormones and breast cancer, gene environment interactions and benign breast disease. It was attended by Heather Baer, Heather Eliassen and Dr. Partridge and Dr. Nekhlyudov, along with other breast cancer researchers. This past four years Dr. Colditz again organized and led this course. Topics in the past covered mathematical models of breast carcinogenesis, associations between endogenous and exogenous hormones and breast cancer, histopathology of benign and malignant breast conditions, estrogen receptivity of tumors, breast morphology (mammographic density), mechanisms of chemoprevention and public health implications of such a strategy, lifestyle factors, (diet and physical activity) and breast cancer, mammographich screening and risk communication. This spring seminar has been established and will evolve into discussions on specific topics on regarding other cancers.

Key Research Accomplishments in Reference to the Statement of Work

- We have successfully recruited and trained four doctoral fellows whom are continuing in the field of breast cancer epidemiology. Heather Baer, Sc.D. Heather Eliassen, ScD., Sonia (Matthews) Maruti, and Jean Marie Eby.
- We have successfully recruited four physician trainees. All four now have M.P.H. degrees and have focused their research training in breast cancer epidemiology. Anne Partridge, M.D., M.P.H. Larissa Nekhlyudov, M.D., M.P.H. Candice Aitken, M.D., M.P.H., Erica Mayer, M.D., M.P.H.
- The Advanced Cancer Epidemiology Seminar in Breast Cancer was established as a result of this award. It continues to be offered as an advanced seminar in breast cancer every spring at Harvard School of Public Health.

Reportable Outcomes

- Dr. Anne Partridge presented orally at the June 2001 American Society of clinical Oncology (ASCO) meeting and won a Merit Scholarship from ASCO for the abstract entitled "Non-adherence with Adjuvant Tamoxifen Therapy in Women with Early Stage Breast Cancer"
- Dr. Partridge received an ASCO Young Investigator's Award for a project to be conducted entitled "Oncologists, practices, preferences, and attitudes regarding providing clinical trial participants feedback on the results of the trials".
- Dr. Partridge published papers entitled "Adherence to therapy with oral antineoplastic agents."¹⁸ and "Non-adherence with adjuvant tamoxifen therapy in women with early stage breast cancer"¹⁹ "Informing clinical trial participants about study results"²⁰ and "Should patients with cancer be offered aggregate results of clinical trials in which they have participated?"²¹
- Dr. Larissa Nekhlyudov presented results to the Society of General Internal Medicine, May 2004 entitled "Effects of Ductal Carcinoma in Situ on Quality of Life: Results from the Nurses' Health Study"¹.
- Dr. Nekhlyudov is the lead investigator on two studies, one addresses screening mammography among women in their 40's, the other is aiming to improve the management of breast symptoms by primary care providers. She is also co-investigator on two NCI funded studies determining the predictors of DCIS recurrence and patient-oriented outcomes of prophylactic mastectomy.
- Dr. Candice Aitken received her M.P.H. June 2004.
- Dr. Aitken has written a protocol and has been IRB approved to do a study that seeks to use stereostatic body radiotherapy in the treatment of liver metastases. She attended the 2nd and 3rd annual SBRT meetings to learn about the work being performed by other groups in this area of research. She will be attending a training session in Wurzburg, Germany to use the stereostatic body frame and treat patients with this highly conformal technique.
- Dr. Aitken has co-written a review article summarizing the current literature on sphincter-sparing therapy for rectal cancer².
- Dr. Aitken presented a poster to the American Society for Therapeutic Radiation Oncologists (ASTRO) evaluating endorectal MRI in a PSA-screened population³.
- Dr. Erica Mayer completed her M.P.H. while a trainee in the last year.
- Dr. Mayer has four IRB protocols at the Dana Farber Cancer Institute which are focused on emerging therapies for treating breast cancer.
- Ms Heather Baer published a paper as first author on adolescent diet and benign breast disease⁴.
- Ms. Baer submitted an abstract regarding body fatness at young ages and incidence of premenopausal breast cancer⁵.
- Ms. Baer was a contributing author on a paper concerning diet and benign breast disease⁶.
- Ms. Baer also contributed to a book chapter⁷.
- Ms. Baer presented a poster at the Harvard Breast Cancer Research Symposium, April 9, 2004 and orally at the Nurses' Health Study External Advisory

Continued -Reportable Outcomes

Committee Meeting, April 21, 2004 and orally at the Society of Epidemiologic Research annual meeting, June 18, 2004 : "Body fatness at young ages and incidence of premenopausal breast cancer".

- Ms. Heather Eliassen submitted an abstract which addresses the association between the use of the statin family of lipid-lowering drugs and breast cancer⁸.
- Ms. Eliassen wrote a second paper which addresses the relation of tubal sterilization with incidence of breast cancer. She submitted an abstract on this topic to the annual meeting of the Society for Epidemiologic Research⁹ held in June 2004.
- Ms. Eliassen also began analyses on the association between weight gain and loss in adult life and incidence of breast cancer.
- Ms. Eliassen is also second author on two papers entitled "Plasma prolactin concentrations and risk of postmenopausal breast cancer"¹⁴ and "Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women"¹⁵.
- Ms. Sonia (Matthews) Maruti had an abstract accepted to "Breast Cancer Research at Harvard Symposium"¹².
- Ms. Maruti is first author on a paper entitled "Adult recall of adolescent diet: reproducibility and comparison with maternal reporting"¹³.
- Ms. Maruti has also submitted a manuscript as first author entitled "Validation of Recalled Adolescent Diet by Adults"¹⁶.
- Ms. Maruti has also submitted an abstract entitled "Validation of Recalled Adolescent Diet by Adults"¹⁷.
- Ms. Maruti has also completed the data collection for our evaluation of our food frequency assessment and is currently working to collect data for a nested case-control study (n~2000) to assess the extent of recall and selection bias in the relation between physical activity and breast cancer.
- Ms. Jean Marie Eby has completed two years of coursework for the doctoral program and passed her written qualifying exam. In preparation for the exam she took classes in epidemiologic methods, biostatistics, cancer epidemiology, biology and cancer prevention. She is beginning to focus her on her dissertation research.
- Ms. Eby has two research projects underway: A nested case control study in the Nurses' Health Study cohort investigating the relationship between C-reactive protein and breast cancer. The second is a prospective cohort analysis of the association between aspirin/NSAIDS use and hip fractures using the Nurses' Health Study data.

Conclusion

Our trainees in breast cancer epidemiology and prevention have proven to be exceptional researchers. As a result of this award, trainees have graduated with advanced degrees in epidemiology from Harvard School of Public Health and the resources of the on-going epidemiologic research at the Brigham and Women's Hospital provided excellent training opportunities for more in-depth breast cancer epidemiology and prevention. As a result of this award, we have achieved our goal of training professionals in translational research.

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Appendices

Plasma Prolactin Concentrations and Risk of Postmenopausal Breast Cancer

Shelley S. Tworoger,^{1,2} A. Heather Eliassen,^{1,2} Bernard Rosner,^{1,3} Patrick Sluss,⁴ and Susan E. Hankinson^{1,2}

¹Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; Departments of ²Epidemiology and ³Biostatistics, Harvard School of Public Health; and ⁴Reproductive Endocrinology Unit Laboratory, Massachusetts General Hospital, Boston, Massachusetts

ABSTRACT

Prolactin is important in human breast development, and substantial laboratory and *in vitro* data suggest a role in mammary carcinogenesis. Therefore, we conducted a prospective case-control study nested within the Nurses' Health Study cohort to examine, in detail, the association between plasma prolactin concentrations and postmenopausal breast cancer by cancer invasiveness, estrogen receptor/progesterone receptor status, and other subject characteristics, including postmenopausal hormone use. Blood samples were collected from 1989 to 1990 and prolactin was measured by microparticle enzyme immunoassay. The analysis included 851 cases of postmenopausal breast cancer diagnosed after blood collection and before June 2000, in which there were one or two controls ($n = 1,275$) matched on age, postmenopausal hormone use, fasting status, and time of day and month of blood collection. Prolactin was associated with a modestly increased risk of postmenopausal breast cancer [relative risk, top *versus* bottom quartile, 1.34; 95% confidence interval (CI), 1.02-1.76; P -trend = 0.01]. The association differed by estrogen receptor/progesterone receptor status (P -heterogeneity = 0.03). The relative risk was 1.78 (95% CI, 1.28, 2.50; P -trend < 0.001) for estrogen receptor+/progesterone receptor+, 0.76 (95% CI, 0.43, 1.32; P -trend = 0.28) for estrogen receptor-/progesterone receptor-, and 1.94 (95% CI, 0.99, 3.78; P -trend = 0.12) for estrogen receptor+/progesterone receptor- breast cancers. Associations generally were similar for ductal and lobular carcinomas (P -heterogeneity = 0.43) and by tumor size (P -heterogeneity = 0.24). Among estrogen receptor+/progesterone receptor+ cancers, the association did not significantly differ by postmenopausal hormone use, years between blood draw and diagnosis, or after adjustment for estradiol (relative risk, 1.93; 95% CI, 1.16, 3.22; P -trend = 0.01). Our prospective data suggest that plasma prolactin concentrations are associated with an increased risk of postmenopausal breast cancer, particularly for estrogen receptor+/progesterone receptor+ cancers, and independently of estradiol.

INTRODUCTION

Prolactin and other sex hormones, such as estradiol and progesterone, are important in normal mammary gland growth and development, as well as lactation (1). Both animal and *in vitro* data suggest that prolactin is involved in tumorigenesis (2) by promoting cell proliferation (3-5), increasing cell motility (6), and improving tumor vascularization (2, 7). Whereas prolactin and its receptor are found in normal and malignant tissues, concentrations of both are generally higher in malignant tissue (5, 8-10).

Epidemiologic data are somewhat limited. In the initial report from the Nurses' Health Study, we reported that postmenopausal women in the highest quartile of prolactin concentrations had an increased risk of breast cancer compared with those in the lowest quartile (relative risk = 2.03; P -trend = 0.01) among 306 breast cancer cases over 4 years of follow-up (11). Two small prospective studies ($n = 26$ and 40

cases) reported a nonsignificantly increased breast cancer risk with higher prolactin concentrations (12, 13). Manjer *et al.* (14) reported no consistent association across quartiles of prolactin, with an odds ratio in the top *versus* bottom quartile of 1.34 [95% confidence interval (CI), 0.83-2.17] among 173 cases. Results of case-control studies have been conflicting, likely because of small sample sizes and the probable influence of breast cancer on prolactin concentrations (2).

Previous studies have not been large enough to consider whether the association may differ among various subgroups of breast cancer or by other subject characteristics. Therefore, we conducted a prospective case-control study nested within the Nurses' Health Study cohort to examine, in further detail, the association between plasma prolactin concentrations and postmenopausal breast cancer by cancer invasiveness, estrogen/progesterone receptor status, and other subject characteristics, including postmenopausal hormone use and antidepressant use. This study includes an additional 445 postmenopausal breast cancer cases compared with our previous report (11).

MATERIALS AND METHODS

Study Population. The Nurses' Health Study cohort was established in 1976 when 121,700 United States female registered nurses, ages 30 to 55 years, completed and returned a mailed questionnaire. The Nurses' Health Study cohort has been followed every 2 years since inception by questionnaire to update exposure variables and ascertain newly diagnosed disease. Data have been collected on various breast cancer risk factors such as weight, height, age at menarche, parity, age at first birth, age at menopause, postmenopausal hormone use, and family history of breast cancer.

Between 1989 and 1990, 32,826 cohort members provided blood samples; women were between 43 and 69 years of age at blood collection. Details about the blood collection methods have been published previously (15). Briefly, women arranged to have their blood drawn and shipped with an ice pack, via overnight courier, to our laboratory where it was processed and separated into plasma, red blood cell, and white blood cell components. Seventy percent of samples were collected while fasting for >8 hours, and 97% were received within 26 hours of collection. The stability of prolactin in whole blood for 24 to 48 hours has been shown previously (16). Samples have been stored in continuously monitored, liquid nitrogen freezers since collection. At blood collection, women completed a short questionnaire asking about current weight, postmenopausal hormone use, and the use of antidepressant medication. Follow-up of the blood study cohort was 99% in 2000.

Both cases and controls were postmenopausal at the time of blood collection. Women were considered to be postmenopausal if they: (1) reported having a natural menopause (e.g., no menstrual cycles during the previous 12 months), (2) had a bilateral oophorectomy, or (3) had a hysterectomy but had at least one ovary remaining, and were at least 56 (for nonsmokers) or 54 (for smokers) years of age (11). These were the ages at which natural menopause occurred for 90% of the overall cohort.

Cases had no reported cancer diagnosis before blood collection and were diagnosed with breast cancer after blood collection but before June 1, 2000. In all, 861 cases of postmenopausal breast cancer, with known postmenopausal hormone status at blood draw, were reported and confirmed by medical record review ($n = 847$) or by verbal confirmation of the diagnosis by the nurse ($n = 14$). Due to the high confirmation rate in medical review (99%), these latter cases were included in the analysis. Time from blood draw to diagnosis ranged from 1 month to 151 months (mean, 67.0 months). Cases and controls were matched on age (± 2 years), recent postmenopausal hormone use, month/year of blood collection (± 1 month), time of day of blood draw (± 2 hours), and fasting status (≥ 10 hours since last meal, <10 hours since last meal, and

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unknown). For cases ($n = 447$) who reported using postmenopausal hormones <3 months before blood collection (*i.e.*, "recent postmenopausal hormone use"), one control was matched per case, and for cases ($n = 414$) who did not report recent postmenopausal hormone use at blood collection, two controls were matched per case; this was done to increase power in analyses only using the latter case group. Exact control subject matches were obtained for 97% of cases on age, 94% on time of day, and 95% on month of blood collection. The most relaxed matches were ± 6 years of age, ± 12 hours, and ± 14 months, respectively. Forty-five control women went on to subsequently develop breast cancer; however, we have only included these individuals as controls.

Reproducibility Study. Among women providing a blood sample in 1989 to 1990, 390 participants were asked to collect two additional samples over the following 2 to 3 years. Participants were postmenopausal, had no prior diagnosis of cancer (except nonmelanoma skin cancer), and had no history of recent postmenopausal hormone use at each sample collection. Of the 390 invited subjects, 186 (48%) sent two additional samples. A random sample of 80 of these women who had all three of the samples drawn between 6 a.m. and 12 p.m. was sent for prolactin analysis and forms the basis of the reproducibility study. Details regarding this study have been published elsewhere (17).

Laboratory Assays. Prolactin was measured using a microparticle enzyme immunoassay. The laboratory of Dr. Christopher Longcope at the University of Massachusetts Medical Center (Boston, MA) assayed 164 cases and 245 controls, in three batches, using the IMx System (Abbott Laboratory, Abbott Park, IL), between March 1993 and August 1997. The remaining samples (697 cases and 1,051 controls) were assayed, in three batches, at the Reproductive Endocrinology Unit Laboratory at the Massachusetts General Hospital, using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL), between August 2001 and November 2003. A subset of 60 samples was assayed at each laboratory; the correlation between the two laboratories was 0.91. The limit of detection (for both laboratories) was 0.6 ng/mL; 1 sample had a value below this limit. Estrone and estradiol were assayed by sensitive and specific radioimmunoassays following organic solvent extraction and Celite column partition chromatography among cases and controls who were not using postmenopausal hormones at blood draw; these methods are described in detail elsewhere (18).

All of the case-control pairs (or triplets) were assayed together, with a random sample order. Laboratory technicians were blinded to case-control status. In each batch we included replicate plasma samples to assess laboratory precision. The intra-assay coefficient of variation ranged from 5.4% to 9.3%. In the final (sixth) batch assayed in 2003 we included 15 control plasma samples from each of the previous five batches, hereafter referred to as drift samples, to assess laboratory drift.

Statistical Analysis. Mean plasma prolactin concentrations from the drift samples differed by batch, indicating that there was some laboratory drift over time. Therefore, using the drift samples, we recalibrated prolactin values from the first five batches to have a comparable distribution to the final batch. To do this we used linear regression, separately by batch, to assess the relationship between the assay value measured in the final batch to that measured in the original batch and used the intercept and β coefficient to rescale all of the values in the original batch. We then created quartile cut points based on all of the controls using the recalibrated prolactin values. Results using these data versus using batch-specific quartile cut points from the original data were very similar; therefore, unless otherwise specified, we present the results using the recalibrated data.

We excluded women who were missing prolactin values related to technical difficulties with the assay ($n = 9$ cases and 16 controls). We identified statistical outliers based on the generalized extreme studentized deviate many-outlier detection approach (19); women with prolactin concentrations >74 ng/mL ($n = 1$ case and 4 controls) or <0.6 ng/mL ($n = 1$ control) were excluded. Overall, 851 cases and 1,275 controls were available for analysis.

For our primary analysis, we used conditional logistic regression to estimate odds ratios and 95% CI comparing quartiles of prolactin concentrations (20). The odds ratios appropriately estimate the relative risks because the outcome is rare; therefore, we henceforth use the term relative risk. In addition, we estimated relative risks and 95% CIs comparing quartiles of prolactin concentrations across various case groups (*in situ* versus invasive, ductal versus lobular, tumor size ≤ 2 cm versus > 2 cm, estrogen receptor/progesterone receptor status, and time between blood draw and diagnosis) using polytomous unconditional logistic regression adjusting for matching factors (21). To de-

termine whether the relative risks across case groups differed, we compared a model holding the association of log-transformed prolactin and breast cancer constant across case groups to one allowing the association to vary, using the likelihood ratio test (21). For time between blood draw and diagnosis, we also conducted a trend test comparing the slopes for log-transformed prolactin concentrations across case groups (22, 23). Secondary, *a priori*, analyses, excluding women with a high prolactin level (>24 ng/mL) or taking antidepressants and stratifying by postmenopausal hormone use used unconditional logistic regression adjusting for matching factors. Using continuous, log-transformed original data (*e.g.*, nonrecalibrated data), we corrected the point and interval estimates for laboratory measurement error and random within-person variation (24). We calculated the within-person variance using the reproducibility study data and the between-person variance using the case-control study data to obtain an intraclass correlation of 0.49.

All of the models were adjusted for the following *a priori* potential confounders: body mass index at age 18 (<21 , 21 – <23 , 23 – <25 , ≥ 25 kg/m², or missing), weight change from age 18 to blood draw (<5 , 5 – <20 , ≥ 20 kg, or missing), family history of breast cancer (yes or no), age at menarche (<12 , 12 , 13 , or ≥ 14 years), age at first birth/parity (nulliparous, age at first birth <25 years/1–4 children, age at first birth 25 – 29 years/1–4 children, age at first birth ≥ 30 years/1–4 children, age at first birth <25 /5 children, or age at first birth ≥ 25 /5 children), and age at menopause (<45 , 45 – 49 , 50 – 54 , or ≥ 55 years). Additional adjustment for oophorectomy, history of benign breast disease, duration of oral contraceptive use, or duration of postmenopausal hormone use did not substantially alter the results. Although we adjusted for parity, which may be part of the biological pathway through which prolactin affects breast cancer (2), it did not alter the risk estimates. Tests for trend were conducted by modeling log-transformed prolactin concentrations continuously and calculating the Wald statistic (25). All of the *P*s were based on two-sided tests and were considered statistically significant if ≤ 0.05 .

RESULTS

Subjects were 45 to 70 years of age (mean, 61 years) at blood collection (Table 1). Differences between cases and controls for age at menarche, age at menopause, parity, and body mass index at age 18 or blood draw generally were small, although in the expected direction. A higher percentage of cases versus controls had a family history of breast cancer (26.0% versus 18.6%, respectively) and a history of benign breast disease (40.5% versus 33.8%, respectively). Cases also had a higher median prolactin concentration than controls ($P < 0.001$).

There was a modest positive association between plasma prolactin concentrations and breast cancer for all of the subjects (P -trend = 0.01; Table 2). The multivariate relative risk in the top versus bottom quartile was 1.34 (95% CI, 1.02, 1.76). This relative risk was slightly attenuated after excluding cases who were diagnosed within 2 years of their blood collection (relative risk, 1.28; 95% CI, 0.98, 1.67; P -trend = 0.02), whereas the relative risk was strengthened slightly (relative risk, 1.39; 95% CI, 1.07, 1.82; P -trend = 0.006) after

Table 1 Characteristics at blood collection of cases and their matched control subjects from the Nurses' Health Study

	Case women ($n = 851$), mean (SD)	Control women ($n = 1,275$), mean (SD)
Age (y)	60.7 (5.1)	61.0 (4.9)
Age at menarche (y)	12.5 (1.4)	12.6 (1.4)
Age at menopause (y)	48.0 (5.6)	47.8 (5.8)
Parity*	3.3 (1.6)	3.4 (1.6)
BMI at age 18 (kg/m ²)	21.2 (2.8)	21.4 (2.9)
BMI at blood draw (kg/m ²)	25.7 (4.8)	25.6 (4.6)
Family history of breast cancer, %	26.0	18.6
History of benign breast disease, %	40.5	33.8
Took anti-depressant medication, %	4.2	4.9
Median prolactin, ng/mL (10 th –90 th percentile)	10.2 (6.2–18.5)	9.4 (6.1–16.7)

Abbreviation: BMI, body mass index.

* Among parous women only.

Table 2 Relative risk (95% CIs) of breast cancer by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study

	n, case/control	Prolactin Concentrations				P for trend*
		≤7.4 ng/mL	>7.4-9.4 ng/mL	>9.4-12.3 ng/mL	>12.3 ng/mL	
Simple relative risk	851/1,275	1.0 (ref.)	0.88 (0.67, 1.16)	1.18 (0.91, 1.53)	1.34 (1.03, 1.74)	0.01
Multivariate relative risk†	851/1,275	1.0 (ref.)	0.90 (0.68, 1.19)	1.18 (0.90, 1.54)	1.34 (1.02, 1.76)	0.01
Excluding cases diagnosed within 2 years of blood draw‡	723/1,275	1.0 (ref.)	0.76 (0.57, 1.01)	1.04 (0.79, 1.36)	1.28 (0.98, 1.67)	0.02
Excluding women with prolactin > 24 ng/mL‡	814/1,232	1.0 (ref.)	0.86 (0.66, 1.12)	1.20 (0.93, 1.56)	1.39 (1.07, 1.82)	0.006

* Determined using continuous, log-transformed prolactin concentrations.

† Adjusted for body mass index at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, and age at menopause.

‡ Adjusted for body mass index at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause and matching factors.

excluding women with a prolactin concentration >24 ng/mL, which is the top end of the normal range.

Results were similar when removing women ($n = 115$ cases and 152 controls) who were taking or unsure if they were taking antidepressant medication, which can alter prolactin concentrations (2), at blood collection (data not shown); comparing the top versus bottom quartile, the relative risk was 1.36 (95% CI, 1.03, 1.80; P -trend = 0.01). Correcting for measurement error and within-person variability, the relative risk increased from 1.28 (95% CI, 1.05, 1.57) to 1.68 (95% CI, 1.10, 2.55) for a one-unit increase in log-transformed prolactin concentrations.

The relationship between plasma prolactin and breast cancer appeared to vary by *in situ* versus invasive cancers (P -heterogeneity = 0.11; Table 3). Overall there was no association for *in situ* cancers (P -trend = 0.84) but a significant positive association for invasive cancers (P -trend = 0.003), with a relative risk of 1.41 (95% CI, 1.08, 1.86) comparing the top to bottom quartiles. The relationship between plasma prolactin and invasive breast cancer was similar for ductal versus lobular types (P -heterogeneity = 0.43) and for tumors ≤2 cm compared with tumors >2 cm (P -heterogeneity = 0.24). However, the risk significantly differed by estrogen receptor and progesterone receptor status (P -heterogeneity = 0.03). The relative risk in the top versus bottom quartile of plasma prolactin was 1.78 (95% CI, 1.28, 2.50; P -trend < 0.001) for estrogen receptor+/pro-

gesterone receptor+ cases, 0.76 (95% CI, 0.43, 1.32; P -trend = 0.28) for estrogen receptor-/progesterone receptor- cases, and 1.94 (95% CI, 0.99, 3.78; P -trend = 0.12) for estrogen receptor+/progesterone receptor- cases. There were too few estrogen receptor-/progesterone receptor+ cases ($n = 18$) to consider separately. Among estrogen receptor+/progesterone receptor+ cases, correcting for laboratory error and within-person variability, the relative risk increased from 1.61 (95% CI, 1.26, 2.06) to 2.77 (95% CI, 1.62, 4.73) for a one-unit increase in log-transformed prolactin concentrations.

Among estrogen receptor+/progesterone receptor+ cases, we found that the association did not differ by postmenopausal hormone use at blood draw (P -interaction = 0.41 comparing past to never users and 0.77 comparing current to never users; Table 4). Although there was no statistical difference by time between diagnosis and blood collection (P -heterogeneity = 0.67), the association appeared to be stronger in the first few years after blood collection; the relative risk for the top versus bottom quartile was 3.10 for 0 to 2 years, 2.23 for 2 to 4 years, 1.56 for 4 to 8 years, and 1.58 for 8+ years (P -trend = 0.12). In a subset of women with measured estrogen concentrations, the relative risk was essentially unchanged compared with all of the women with estrogen receptor+/progesterone receptor+ tumors. Additional adjustment for estrone or estradiol concentrations also did not alter the results. Results were similar when considering all

Table 3 Multivariate* relative risk (95% CI) of breast cancer by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study by invasiveness, type, tumor size, and receptor status†

	Prolactin Concentrations				P for trend‡	P for heterogeneity§
	≤7.4 ng/mL	>7.4-9.4 ng/mL	>9.4-12.3 ng/mL	>12.3 ng/mL		
<i>In situ</i> ($n = 115$ cases)	1.0 (ref.)	0.85 (0.49, 1.46)	0.80 (0.46, 1.39)	0.96 (0.57, 1.63)	0.84	0.11
Invasive ($n = 722$ cases)	1.0 (ref.)	0.84 (0.63, 1.12)	1.27 (0.97, 1.67)	1.41 (1.08, 1.86)	0.003	
Ductal ($n = 588$ cases)	1.0 (ref.)	0.85 (0.62, 1.15)	1.19 (0.89, 1.60)	1.38 (1.04, 1.85)	0.007	0.43
Lobular ($n = 93$ cases)	1.0 (ref.)	0.86 (0.42, 1.76)	1.69 (0.90, 3.14)	1.76 (0.95, 3.26)	0.11	
Tumor size ≤2 cm ($n = 531$ cases)	1.0 (ref.)	0.87 (0.63, 1.19)	1.22 (0.91, 1.65)	1.35 (1.00, 1.83)	0.01	0.24
Tumor size >2 cm ($n = 162$ cases)	1.0 (ref.)	0.74 (0.43, 1.28)	1.35 (0.84, 2.19)	1.66 (1.04, 2.64)	0.03	
ER+/PR+ ($n = 397$ cases)	1.0 (ref.)	0.87 (0.60, 1.26)	1.52 (1.08, 2.13)	1.78 (1.28, 2.50)	<0.001	0.03
ER-/PR- ($n = 96$ cases)	1.0 (ref.)	0.49 (0.26, 0.92)	0.73 (0.41, 1.28)	0.76 (0.43, 1.32)	0.28	
ER+/PR- ($n = 91$ cases)	1.0 (ref.)	1.76 (0.90, 3.47)	1.74 (0.88, 3.43)	1.94 (0.99, 3.78)	0.12	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index.

* Adjusted for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause, and matching factors.

† Too few ER-/PR+ cases ($n = 18$) were available to analyze separately.

‡ Determined using continuous, log-transformed prolactin concentrations.

§ Determined using polytomous logistic regression and the likelihood ratio test, comparing a model constraining relative risks to be the same across all case groups versus a model allowing the relative risks to differ across case groups.

Table 4 Multivariate* relative risks (95% CI) for ER+/PR+ breast cancers by quartile of plasma prolactin concentration among postmenopausal women in the Nurses' Health Study

	n, case/control	Prolactin Concentrations				P for trend†
		≤7.4 ng/mL	>7.4–9.4 ng/mL	>9.4–12.3 ng/mL	>12.3 ng/mL	
By postmenopausal hormone use at blood draw‡						
Never user	111/545	1.0 (ref.)	1.04 (0.54, 1.97)	1.74 (0.95, 3.16)	1.89 (1.02, 3.50)	0.16
Past user	67/277	1.0 (ref.)	1.00 (0.42, 2.40)	1.76 (0.82, 3.78)	2.33 (1.06, 5.14)	0.02
Current user	219/453	1.0 (ref.)	0.76 (0.44, 1.31)	1.29 (0.79, 2.12)	1.55 (0.98, 2.47)	0.01
By time between blood draw and diagnosis (for cases)§						
0–2 y	52/1,275	1.0 (ref.)	2.01 (0.68, 5.98)	4.23 (1.57, 11.4)	3.10 (1.11, 8.62)	0.04
2–4 y	74/1,275	1.0 (ref.)	0.77 (0.33, 1.80)	1.63 (0.80, 3.33)	2.23 (1.13, 4.41)	0.02
4–8 y	161/1,275	1.0 (ref.)	0.67 (0.39, 1.16)	1.20 (0.75, 1.94)	1.56 (0.99, 2.47)	0.02
8+ y	110/1,275	1.0 (ref.)	1.05 (0.57, 1.94)	1.33 (0.74, 2.38)	1.58 (0.90, 2.79)	0.03
Adjusting for estrone or estradiol among never and past postmenopausal hormone users						
Not adjusted	175/784	1.0 (ref.)	1.00 (0.59, 1.69)	1.73 (1.06, 2.81)	1.99 (1.20, 3.30)	0.01
Estrone	131/597	1.0 (ref.)	0.82 (0.44, 1.54)	1.64 (0.92, 2.92)	2.18 (1.21, 3.94)	0.02
Estradiol	175/784	1.0 (ref.)	0.98 (0.57, 1.66)	1.77 (1.08, 2.90)	1.93 (1.16, 3.22)	0.01

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index.

* Adjusted for BMI at age 18, weight change from age 18 to blood draw, family history of breast cancer, age at menarche, age at first birth/parity, age at menopause, and matching factors.

† Determined using continuous, log-transformed prolactin concentrations.

‡ The *P*-interaction between past and never users was 0.41 and between current and never users was 0.77.

§ The *P*-heterogeneity was 0.67, determined using polytomous logistic regression and the likelihood ratio test, comparing a model constraining relative risks to be the same across all case groups versus a model allowing the relative risks to differ across case groups.

of the estrogen receptor+ cases together, regardless of progesterone receptor status (data not shown).

DISCUSSION

This is the largest prospective study to date examining the association between plasma prolactin concentrations and postmenopausal breast cancer and the first to evaluate relationships by tumor characteristics. We observed a positive association between prolactin and breast cancer risk overall. However, the increased risk appeared to be confined to invasive cancers, particularly those tumors that were estrogen receptor+/progesterone receptor+ and estrogen receptor+/progesterone receptor-. Also, the association appeared strongest for estrogen receptor+/progesterone receptor+ cases diagnosed within 2 years of blood collection, although we still observed a positive association among tumors diagnosed 8 or more years after blood collection.

The overall positive association that we observed between plasma prolactin and breast cancer generally is consistent with previous studies. In an earlier analysis of this same dataset, consisting of 306 cases, the relative risk comparing the top to bottom quartile was stronger than in the present study (relative risk, 2.03; 95% CI, 1.24, 3.31); the risk was slightly stronger after excluding *in situ* cancers (11), which is similar to the results of the present study. Wang *et al.* (13) reported a nonsignificant increased breast cancer risk comparing the top to the bottom quintile of prolactin, with a relative risk of 1.63 (95% CI, 0.57, 4.71); Kabuto *et al.* (12) also reported a nonsignificant increased risk. The lack of statistical significance in both studies is probably due to the small number of cases available for analysis (*n* = 40 and 26 cases, respectively). One prospective nested case-control study with 173 *in situ* and invasive cases and 438 controls found no consistent association after adjustment for matching factors and body mass index (14). However, the odds ratio in the top versus bottom quartile (1.34; 95% CI, 0.83, 2.17) was similar to the relative risk that we observed when including all of the cases.

Both animal and *in vitro* models support the hypothesis that prolactin is involved in mammary carcinogenesis. Several studies have reported that breast cancer cells/tissue express prolactin (9, 26–28) and the prolactin receptor (8–10, 28). Although normal tissue also expresses the prolactin receptor, primarily along the luminal cell border, several studies have reported higher levels in tumor tissue (10, 29) with expression primarily in the cytoplasm (8). In mice, prolactin appears to induce tumor formation (30, 31), increase tumor growth rate (5), and increase the number of cells in the S phase (31). *In vitro* studies also suggest that prolactin is associated with higher cell proliferation rates (3–5), increases in cyclin D1 (3, 4), and it may induce motility of breast cancer cell lines (6). Preliminary data also suggest that prolactin can enhance the responsiveness of breast cancer cells to estradiol (3). In humans, prolactin concentrations were positively associated with mammographic density, a consistent strong breast cancer risk factor (32), among 189 postmenopausal women after adjustment for age and waist circumference (33). Paradoxically, prolactin is temporarily increased during breastfeeding, which is a protective factor for breast cancer. One possible explanation for this discrepancy is that pregnancy is associated with a lifetime decrease in prolactin levels (2), and this may outweigh the transient prolactin increase during breastfeeding. Secondly, the effect of prolactin during breastfeeding may differ from its effect at other times in the reproductive life of a woman; for example, it may lead to terminal cell differentiation during lactation but not at other times.

We found that the increased risk of postmenopausal breast cancer associated with high prolactin concentrations was confined primarily to invasive cancers, particularly estrogen receptor+/progesterone receptor+ and estrogen receptor+/progesterone receptor- tumors. Of the multiple functions of prolactin, it is possible that increasing survival and motility are the predominate effects on tumor cells, which would promote increased invasion into the surrounding stromal tissue (2). Several (8, 10, 34–37), but not all (9, 38–40), studies have reported that the prolactin receptor and estrogen receptor are coex-

pressed; results for the coexpression with progesterone receptor are less clear (8, 10, 36, 37, 39, 40). Differences between studies may be due, at least in part, to the different methods of detecting the presence of the prolactin receptor (2, 10). The mechanism underlying coexpression of the prolactin receptor and estrogen receptor is unclear. Several *in vitro* studies have reported that long-term prolactin exposure can increase estrogen receptor expression (3, 10). Rose-Hellekant *et al.* (31) reported that transgenic mice with constant prolactin expression developed both estrogen receptor+ and estrogen receptor- tumors, although estrogen receptor+ tumors are extremely rare in this mouse model. These data taken together suggest that prolactin may be important in the development of estrogen receptor+ tumors.

We found that among estrogen receptor+/progesterone receptor+ tumors, the association of prolactin did not differ by postmenopausal hormone use, although postmenopausal hormone can increase prolactin concentrations (11). This is particularly interesting given that the association between estrogens and postmenopausal breast cancer risk appears to be stronger in never-postmenopausal hormone users compared with past users (18, 41). We also found that prolactin concentrations predict breast cancer risk independently of estrone or estradiol concentrations among never and past users of postmenopausal hormones at blood collection.

Whereas we observed an association between prolactin and estrogen receptor+/progesterone receptor+ breast cancers diagnosed >8 years after blood collection, the association appeared to be strongest among cases diagnosed within 2 years of blood collection. If recent/current levels are most important for risk then the risk estimates will be attenuated in later years, because the within-woman reproducibility decreases over time, introducing increased measurement error. Alternatively, our results are consistent with the possibility that subclinical breast cancer may increase prolactin concentrations. Bhatavdekar *et al.* (28) reported that immunohistochemical staining of prolactin in breast tumors was correlated with plasma prolactin concentrations ($r = 0.41$). Thus, it is important for studies examining the relationship between prolactin and breast cancer to use a prospective design, especially in light of other evidence that prolactin secretion can be influenced by physical or psychological stress after diagnosis (42–44).

This study has several limitations. First, several forms of prolactin circulate in human plasma, which appear to have different biological activities (45, 46). The immunoassay used in this study, which identifies most prolactin isoforms (47), cannot distinguish between them. Therefore, we cannot isolate which isoform or isoforms are most important in breast cancer development. Second, although we used a high-precision assay, there was laboratory drift over time, potentially precluding us from considering the relationship of absolute prolactin concentrations with breast cancer. However, the addition of drift samples in the final batch of assays allowed us to recalibrate previous batches so that we could consider the relative concentrations. Third, prolactin has a strong circadian rhythm (48) and increases after a noontime meal (49). To minimize misclassification related to time of day of blood draw and fasting status, we closely matched case and control subjects on both of these factors. Also, prolactin values tend to fluctuate over time within a woman (intraclass-correlation over 3 years = 0.49) among postmenopausal women (17), which could attenuate the risk estimates. Therefore, we used information from a reproducibility study to correct our relative risk estimates for the random, biological variation in prolactin concentrations that cannot be captured by a single hormone measurement.

This is the largest prospective epidemiologic study of plasma prolactin concentrations and breast cancer, which allowed us to consider whether the risk differed by various subtypes of breast cancer tumors or other subject characteristics. We demonstrated a moderate

positive association between plasma prolactin concentrations and the risk of invasive postmenopausal breast cancer, which is independent of estrogen concentrations. Furthermore, it appears that prolactin is associated primarily with estrogen receptor+ tumors. Our study lends substantial support to the hypothesis that prolactin is important in breast cancer etiology, although additional confirmation in other prospective studies is needed. It is also important to study whether prolactin may be important in the development of premenopausal breast cancer.

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Endogenous Estrogen, Androgen, and Progesterone Concentrations and Breast Cancer Risk Among Postmenopausal Women

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Background: Levels of endogenous hormones have been associated with the risk of breast cancer among postmenopausal women. Little research, however, has investigated the association between hormone levels and tumor receptor status or invasive versus *in situ* tumor status. Nor has the relation between breast cancer risk and postmenopausal progesterone levels been investigated. We prospectively investigated these relations in a case-control study nested within the Nurses' Health Study. **Methods:** Blood samples were prospectively collected during 1989 and 1990. Among eligible postmenopausal women, 322 cases of breast cancer (264 invasive, 41 *in situ*, 153 estrogen receptor [ER]-positive and progesterone receptor [PR]-positive [ER⁺/PR⁺], and 39 ER-negative and PR-negative [ER⁻/PR⁻] disease) were reported through June 30, 1998. For each case subject, two control subjects (n = 643) were matched on age and blood collection (by month and time of day). Endogenous hormone levels were measured in blood plasma. We used conditional and unconditional logistic regression analyses to assess associations and to control for established breast cancer risk factors. **Results:** We observed a statistically significant direct association between breast cancer risk and the level of both estrogens and androgens, but we did not find any (by year) statistically significant associations between this risk and the level of progesterone or sex hormone binding globulin. When we restricted the analysis to case subjects with ER⁺/PR⁺ tumors and compared the highest with the lowest fourths of plasma hormone concentration, we observed an increased risk of breast cancer associated with estradiol (relative risk [RR] = 3.3, 95% confidence interval [CI] = 2.0 to 5.4), testosterone (RR = 2.0, 95% CI = 1.2 to 3.4), androstenedione (RR = 2.5, 95% CI = 1.4 to 4.3), and dehydroepiandrosterone sulfate (RR = 2.3, 95% CI = 1.3 to 4.1). In addition, all hormones tended to be associated most strongly with *in*

situ disease. **Conclusion:** Circulating levels of sex steroid hormones may be most strongly associated with risk of ER⁺/PR⁺ breast tumors. [J Natl Cancer Inst 2004;96:1856-65]

Epidemiologic data now provide strong evidence for an influence of plasma steroid hormones on the risk of breast cancer in postmenopausal women (1)—a long proposed, but previously poorly supported, hypothesis. The associations between the risk of breast cancer and the level of estrogens and androgens (with relative risks [RRs] for breast cancer ranging from 2.0 to 2.5 when comparing the top 20% with the bottom 20% of hormone levels) are strong compared with those of most other breast cancer risk factors. However, few studies have investigated these associations as stratified by tumor receptor status or by invasive versus *in situ* disease. In addition, studies of the effect of postmenopausal hormone use suggest that formulations containing estrogen and progestin are associated with a greater increase in breast cancer risk than those with estrogen only (2-5). However, the influence of endogenous progesterone levels remains unknown.

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Within the large, prospective Nurses' Health Study cohort, we previously investigated (6) the relation between endogenous estrogens and androgens and breast cancer risk among postmenopausal women (156 cases of breast cancer with follow-up from 1990 through 1994) and found. To explore the association between endogenous hormone levels and breast cancer risk in greater detail than was previously possible, we conducted a second nested case-control study that extends the follow-up through 1998 and increases the total number of incident cases of breast cancer to 322. We evaluated the associations between endogenous hormone levels and breast cancer risk overall and assessed whether the associations varied by stratification by other breast cancer risk factors, by tumor receptor status, or by invasive versus *in situ* disease.

MATERIALS AND METHODS

Study Population

The Nurses' Health Study cohort was established in 1976 when 121 700 female registered nurses, 30–55 years of age, completed and returned a mailed questionnaire. The cohort continues to be followed every 2 years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on most known breast cancer risk factors including height, weight, age at menarche and menopause, age at first birth, postmenopausal hormone use, and family history of breast cancer.

During 1989 and 1990, blood samples were collected from 32 826 cohort members, who were 43–69 years of age at blood collection and formed the blood cohort. Details regarding the blood collection methods have been previously published (6,7). Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier and with an ice-pack, to our laboratory, where it was processed and separated into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored liquid nitrogen freezers since collection. As of 1998, the follow-up rate among the women who provided blood samples was 99%.

Both case and control subjects in this analysis are women who, at blood collection, were postmenopausal and had not used postmenopausal hormones for at least 3 months. Of the blood cohort, 11 169 women met these criteria; case and control subjects were selected from this sub-cohort. We defined a postmenopausal participant in this study as a woman who reported having a natural menopause or a bilateral oophorectomy or as a woman who reported having a hysterectomy with either one or both ovaries remaining when she was 56 years old (if a non-smoker) or 54 years old (if a current smoker), ages at which natural menopause had occurred in 90% of these respective groups.

Case subjects in this analysis are women with no reported cancer diagnosis (other than non-melanoma skin cancer) before blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1998. Overall, 322 cases of breast cancer (264 invasive, 41 *in situ*, 153 estrogen receptor (ER)- and progesterone receptor (PR)-positive [ER⁺/PR⁺], and 39 ER-negative and PR-negative [ER⁻/PR⁻] disease) were reported from among the 11 169 women eligible at baseline. All cases of breast cancer were confirmed by and tumor details (receptor status and invasive versus *in situ* tumors) were ob-

tained from a medical record review, with one exception. A single nurse confirmed the diagnosis of breast cancer, but the medical record was unavailable. Because of the high confirmation rate upon medical record review (99%) in the Nurses' Health Study, we kept this case subject in the analysis. However, 17 cases were not included in the invasive versus *in situ* case sub-analyses because the pathology report was unclear as to whether the tumor was invasive or because the information was missing. Time from blood collection to diagnosis ranged from less than 1 month to 106 months (median = 52 months; 5th percentile–95th percentile = 4–96 months). Two control subjects (total n = 643) were matched per case subject by age (year), month of blood collection, time of day that blood was drawn (± 2 hours), and fasting status at the time of blood collection (≥ 10 hours since a meal versus < 10 hours or unknown). Ninety-four percent of control matches were exact; the most relaxed matches were within ± 6 years of age, ± 14 months of blood collection from case subjects, and ± 11 hours for time of blood collection. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Laboratory Analyses

Analyses were conducted by three different laboratories. For estrone, estradiol, androstenedione, testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS), all batches were assayed at Quest Diagnostic's Nichols Institute (San Juan Capistrano, CA). For estrone sulfate, the first batch was assayed at the University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory (Worcester); the remaining batches were assayed at Nichols. The first two batches of sex hormone-binding globulin (SHBG) were assayed at the Longcope Laboratory; the third and fourth batches were assayed at Massachusetts General Hospital's Reproductive Endocrinology Unit Laboratory (Boston). All batches of progesterone were assayed at the same time at Quest Diagnostics.

Hormone assay methods have been described previously in detail (6). Endogenous hormone levels were measured in blood plasma. In brief, samples were extracted with a mixture of hexane and ethyl acetate (4:1, vol/vol) and applied to a celite column, the steroids were eluted from the column (celite in ethylene glycol), and the fractions were subjected to radioimmunoassay (8–12). DHEAS was assayed by radioimmunoassay without a prior separation step (13). To quantify estrone sulfate levels, estrone was first extracted from the plasma, and then the estrone sulfate bond was enzymatically cleaved to release estrone, which was then extracted from the plasma by an organic solvent and was subjected to chromatography and then radioimmunoassay (14). Free and percent free estradiol were calculated by the law of mass action according to the method described by Sodergard et al. (15).

All case-control-control triplet samples were assayed together; the samples were ordered randomly within a triplet and labeled so that the laboratory could not identify the case-control status. Although all members of a triplet were analyzed at the same time, the triplets were analyzed in up to five different batches (sent in 1992, 1993, 1996, 1998, and 2001). To assess laboratory precision, replicates of 10% of all samples assayed were randomly interspersed and labeled to preclude their iden-

tification. Within-batch laboratory coefficients of variation ranged from 6% (DHEAS) to 15% (progesterone).

The detection limits of the assays were as follows: 2 pg/mL for estradiol, 10 pg/mL for estrone, 40 pg/mL for estrone sulfate (in each laboratory), 3 ng/dL for androstenedione, 1 ng/dL for testosterone, 3 ng/dL for DHEA, 5 µg/dL for DHEAS, and 3 ng/dL for progesterone. When plasma hormone values were reported as less than the detection limit, we set the value to half this limit. Values were less than the detection limit of estrone in 22 samples, estrone sulfate in three samples, androstenedione in one sample, testosterone in two samples, DHEA in one sample, DHEAS in five samples, and progesterone in 274 samples.

Covariate Data

We obtained information on other breast cancer risk factors from one or more of the biennial NHS questionnaires. Age at menarche and height were asked on the 1976 questionnaire. Age at first birth and parity were asked on the 1976 questionnaire and updated until the 1984 questionnaire. Family history of breast cancer was asked on the 1976 questionnaire and updated on the 1982 and 1988 questionnaires. Weight at age 18 years was asked on the 1980 questionnaire; current weight was obtained from the questionnaire completed at blood collection. Menopausal status and postmenopausal hormone use was asked on all biennial questionnaires, and this information was updated until diagnosis of breast cancer when case subjects were identified and matched to control subjects.

Statistical Analyses

We used quartile cut points to divide the data into fourths, with cut points based on the distribution in the control subjects. For most of the hormones, we chose quartile cut points according to the distribution in the control subjects overall and used the lowest fourth as the referent in all analyses.

For estrone, estrone sulfate, testosterone, estradiol, and DHEA, the median value for the control subjects varied in such a way that quartile cut points that were based on all control subjects combined resulted in uneven batch-specific distributions (between batch differences in medians ranged from $\leq 2\%$ up to a maximum of 30%-60% depending on the hormone). Because the mean value of the quality-control replicates in each

of the datasets varied in the same manner for these five assays, much (if not all) of this difference appeared to be caused by laboratory drift rather than by true differences in hormone levels between the batches. When the fifth batch was sent for assay of estrone, estrone sulfate, testosterone, and DHEA, we included approximately 10 samples from each of the previous batches to assess laboratory drift. Using the mean percent change between each of the first four batches and the fifth batch, we recalibrated the earlier hormone values to the fifth batch scale. Thus, for these four hormones, we defined one set of batch-specific quartile cut points by the recalibrated values combined with the fifth batch values. For estradiol, the median value for the control subjects in the fifth batch varied from the first four batches by 50%, but we had not included samples from earlier batches to allow recalibration. Thus, for estradiol, we defined two quartile cut points: one that was based on the first four batches combined and the other that was based on the fifth batch. We also controlled for batch in all analyses. When statistical analyses were repeated with batch-specific cut points for all hormones, rather than the recalibrated data, results were nearly identical.

We removed two matched sets of a case subject and two control subjects from the analysis, because the case subject's estrogen values were in the premenopausal range, dropping the total number of case subjects from 324 to 322. We used the extreme Studentized deviate Many-Outlier procedure (16,17) to assess for outliers in each set of laboratory results. This procedure resulted in the removal of three estradiol values, four androgen values, two testosterone values, one DHEA value, and three progesterone values. In addition, several women did not have a sufficient volume of plasma for all assays. Therefore, from the 322 total case subjects, the final number of case and control samples available for each individual hormone analysis is shown in Table 1. Case subject and control subject distributions across the data by fourths for each individual hormone are shown in Table 2.

To test for differences in hormone levels between case subjects and control subjects, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and to adjust for any residual correlation between case subjects and control subjects within the matched set (18). To maintain matched triplet integrity, we used conditional logistic regression to estimate odds ratios (referred to

Table 1. Plasma hormone levels for postmenopausal case subjects and matched control subjects

Hormone	Case definition								<i>P</i> value†
	Control subjects		All case subjects		Case subjects with invasive disease		Case subjects with <i>in situ</i> disease		
					No.	Median (range*)	No.	Median (range*)	
Estradiol, pg/mL	637	6 (4–13)	319	7 (4–15)	261	7 (4–15)	41	8 (5–17)	<.001
Free estradiol, pg/mL	605	0.10 (0.05–0.21)	301	0.11 (0.05–0.26)	247	0.11 (0.05–0.27)	39	0.10 (0.06–0.24)	<.001
Estrone, pg/mL	624	23 (14–38)	320	26 (15–43)	262	26 (15–43)	41	28 (15–45)	<.001
Estrone sulfate, pg/mL	622	280 (136–600)	313	339 (154–823)	258	339 (154–823)	39	348 (150–823)	<.001
Progesterone, ng/dL	530	4.0 (1.5–10.0)	270	4.0 (1.5–10.0)	222	4.0 (1.5–10.0)	32	4.0 (1.5–10.5)	.64
Sex hormone binding globulin, nm/L	622	48 (24–79)	310	47 (21–81)	255	44 (20–80)	39	56 (22–87)	.08
Androstenedione, ng/dL	621	57 (31–103)	312	62 (38–103)	255	61 (37–103)	41	63 (39–99)	.01
Testosterone, ng/dL	628	19 (11–33)	312	22 (12–37)	256	22 (12–37)	40	22 (14–39)	<.001
Free testosterone, ng/dL	608	0.22 (0.10–0.43)	301	0.25 (0.13–0.51)	248	0.25 (0.13–0.52)	38	0.25 (0.14–0.46)	<.001
Dehydroepiandrosterone, ng/dL	603	248 (116–473)	305	283 (127–557)	248	258 (133–564)	41	328 (122–536)	.01
Dehydroepiandrosterone sulfate, µg/dL	634	85 (35–169)	320	90 (44–205)	262	90 (43–200)	41	90 (50–205)	<.001

*Range from median of the bottom fourth (12.5%) to median of the top fourth (87.5%).

†P value, from the mixed-effects regression model comparing all case subjects to control subjects, controlling for matching factors; two-sided.

Table 2. Quartile ranges for plasma hormone levels among postmenopausal case subjects and matched control subjects

Plasma hormone	Quartile ranges (No. case subjects/No. control subjects)			
Estradiol, pg/mL	*	*	*	*
	(79/209)	(82/168)	(49/116)	(109/144)
Free estradiol, pg/mL	<0.064	0.064–0.096	0.097–0.148	>0.148
	(55/149)	(81/155)	(59/147)	(106/154)
Estrone, pg/mL	<18	18–23	24–30	>30
	(68/159)	(67/160)	(79/151)	(106/154)
Estrone sulfate, pg/mL	<178	178–279	280–421	>421
	(50/155)	(76/155)	(70/157)	(117/155)
Progesterone, ng/dL	<1.6	1.6–4.0	4.1–8.0	>8.0
	(91/191)	(49/95)	(78/139)	(52/105)
SHBG, nm/L	<34	34–48	49–67	>67
	(88/150)	(72/166)	(75/149)	(75/157)
Androstenedione, ng/dL	<43	43–57	58–78	>78
	(64/159)	(64/156)	(93/155)	(91/151)
Testosterone, ng/dL	<15	15–19	20–26	>26
	(66/164)	(54/153)	(95/160)	(97/151)
Free testosterone, ng/dL	<0.16	0.16–0.22	0.23–0.32	>0.32
	(54/156)	(71/154)	(86/145)	(90/153)
DHEA, ng/dL	<165	165–247	248–367	>367
	(67/152)	(69/149)	(74/150)	(95/152)
DHEAS, µg/dL	<52	52–85	86–135	>135
	(53/160)	(96/162)	(81/156)	(90/156)

*Batch-specific quartile cut points were used to categorize estradiol. The cut points for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cutpoints for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL. SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate.

herein as relative risks) and 95% confidence intervals (CIs) in the total data set (19). Results from simple and multivariable models were very similar. To increase statistical power, we used unconditional logistic regression, controlling for the matching factors, for all subset analyses (e.g., analyses according to prior postmenopausal hormone use or tumor receptor status or analyses that were stratified by invasive versus *in situ* cases). These analyses thus include case subjects and control subjects whose match was excluded because of outlying hormone values (as described above) or missing sub-group-defining data. These subset analyses also were conducted by conditional logistic regression, and results were similar, although less precise. We conducted tests for trend by modeling the natural logarithm of the hormone level as a continuous variable and calculating a Wald statistic (19). Additionally, we calculated tests for trend by modeling the median of the fourths of each hormone. All *P* values were from two-sided tests.

To test for differences in trend across fourths of hormone level by breast cancer tumor characteristics, we used polychotomous logistic regression (20) with three end points for tumor invasiveness (invasive, *in situ*, and no breast cancer) and four end points for tumor receptor status (ER⁺/PR⁺, ER⁺/PR⁻, ER⁻/PR⁻, and no breast cancer). One and two degree of freedom tests, respectively, compared a model with separate slopes in each ER/PR group to a model with a common slope. The likelihood ratio test statistic was applied to a chi-squared distribution to obtain two-sided *P* values. Too few cases of ER⁺/PR⁺ disease occurred (*n* = 6) in the cohort for this tumor receptor pattern to be considered separately.

The interactions between hormone levels and established breast cancer risk factors were evaluated by adding cross-classified variables (e.g., estrone [medians of continuous

fourths] × postmenopausal hormone use [dichotomized as never and past, which was defined by use up to time of diagnosis or control referent date]) to the logistic models; presence of an interaction was assessed with the Wald test. These analyses were conducted among all women combined.

RESULTS

Both case subjects and control subjects in this analysis ranged in age from 45 to 69 years, with a mean age of 62 years. The mean years since menopause (13.2 versus 13.5 years), body mass index at age 18 years (21.4 versus 21.6 kg/m²), parity (3.2 versus 3.3 children), age at first birth (25.7 versus 25.4 years), and age at menopause (48.7 versus 48.4 years) did not differ between case subjects and control subjects, respectively. Case subjects, compared with control subjects, were statistically significantly more likely to have a family history of breast cancer (24.2% versus 17.1%; *P* = .01) and were younger at menarche (12.5 versus 12.7 years; *P* = .03). Circulating steroid hormone levels were statistically significantly greater among case subjects with breast cancer than among control subjects for all hormones investigated, with the exception of progesterone (Table 1). In conditional logistic regression models that were adjusted for known breast cancer risk factors (body mass index at age 18 years, family history of breast cancer, age at menarche, age at first birth, parity, age at menopause, and duration of postmenopausal hormone use), the risk of breast cancer was statistically significantly greater among the highest fourth than among the lowest fourth and was linearly associated across fourths for all hormones, except for progesterone and SHBG (Table 3). When tests for trend were modeled with the median of the fourths for each hormone, results were nearly identical, except for trends

Table 3. Risk of breast cancer by fourths of plasma hormone levels among postmenopausal women

Plasma hormone	RR (95% CI)*				P value†
	1	2	3	4	
Estradiol, pg/mL					
MV RR‡	1.0 (referent)	1.3 (0.9 to 1.9)	1.1 (0.7 to 1.7)	2.1 (1.5 to 3.2)	<.001
Invasive disease	1.0 (referent)	1.4 (0.9 to 2.1)	1.3 (0.8 to 2.0)	2.0 (1.3 to 3.0)	<.001
<i>In situ</i> disease§	1.0 (referent)	1.9 (0.7 to 4.8)	1.1 (0.4 to 3.7)	3.0 (1.2 to 7.4)	.01
Never PMH use	1.0 (referent)	1.6 (0.8 to 3.0)	1.9 (1.0 to 3.7)	3.6 (2.0 to 6.4)	<.001
Past PMH use	1.0 (referent)	1.3 (0.8 to 2.3)	0.8 (0.4 to 1.5)	1.6 (0.9 to 2.8)	.22
Free estradiol, pg/mL					
MV RR‡	1.0 (referent)	1.3 (0.9 to 2.0)	1.0 (0.7 to 1.6)	1.9 (1.2 to 2.9)	<.001
Invasive disease	1.0 (referent)	1.4 (0.9 to 2.3)	1.1 (0.7 to 1.8)	2.0 (1.3 to 3.1)	<.001
<i>In situ</i> disease§	1.0 (referent)	2.4 (0.9 to 6.7)	1.6 (0.5 to 4.8)	2.2 (0.8 to 6.4)	.12
Never PMH use	1.0 (referent)	1.4 (0.7 to 2.8)	1.1 (0.6 to 2.3)	2.6 (1.4 to 4.9)	<.001
Past PMH use	1.0 (referent)	1.3 (0.7 to 2.3)	1.0 (0.5 to 1.9)	1.4 (0.8 to 2.7)	.19
Estrone, pg/mL					
MV RR‡	1.0 (referent)	1.0 (0.7 to 1.6)	1.3 (0.9 to 2.0)	1.7 (1.1 to 2.6)	<.001
Invasive disease	1.0 (referent)	0.9 (0.6 to 1.4)	1.2 (0.8 to 1.8)	1.4 (0.9 to 2.2)	.003
<i>In situ</i> disease§	1.0 (referent)	1.5 (0.5 to 4.4)	1.8 (0.6 to 5.2)	3.0 (1.1 to 8.2)	.01
Never PMH use	1.0 (referent)	0.7 (0.3 to 1.4)	1.6 (0.9 to 3.0)	3.0 (1.7 to 5.5)	<.001
Past PMH use	1.0 (referent)	1.1 (0.6 to 1.9)	1.0 (0.6 to 1.9)	0.8 (0.4 to 1.5)	.72
Estrone sulfate, pg/mL					
MV RR‡	1.0 (referent)	1.6 (1.0 to 2.5)	1.4 (0.9 to 2.2)	2.4 (1.6 to 3.8)	<.001
Invasive disease	1.0 (referent)	1.5 (1.0 to 2.4)	1.3 (0.8 to 2.1)	2.2 (1.4 to 3.5)	<.001
<i>In situ</i> disease§	1.0 (referent)	2.0 (0.6 to 6.4)	2.4 (0.8 to 7.3)	3.5 (1.2 to 10.2)	.03
Never PMH use	1.0 (referent)	1.5 (0.8 to 3.0)	1.9 (1.0 to 3.7)	3.4 (1.8 to 6.3)	<.001
Past PMH use	1.0 (referent)	1.5 (0.8 to 2.7)	1.2 (0.6 to 2.2)	1.8 (1.0 to 3.2)	.06
Progesterone, ng/dL					
MV RR‡	1.0 (referent)	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	0.9 (0.6 to 1.5)	.90
Invasive disease	1.0 (referent)	0.9 (0.5 to 1.4)	1.1 (0.7 to 1.7)	0.8 (0.5 to 1.3)	.77
<i>In situ</i> disease§	1.0 (referent)	2.9 (1.1 to 7.6)	1.2 (0.4 to 3.5)	1.6 (0.5 to 5.0)	.67
SHBG, nm/L					
MV RR‡	1.0 (referent)	0.7 (0.5 to 1.1)	0.9 (0.6 to 1.3)	0.8 (0.6 to 1.3)	.14
Invasive disease	1.0 (referent)	0.7 (0.5 to 1.1)	0.7 (0.5 to 1.1)	0.8 (0.5 to 1.3)	.04
<i>In situ</i> disease§	1.0 (referent)	0.6 (0.2 to 1.9)	2.3 (1.0 to 5.7)	0.6 (0.2 to 1.9)	.76
Androstenedione, ng/dL					
MV RR‡	1.0 (referent)	1.0 (0.7 to 1.6)	1.6 (1.0 to 2.3)	1.5 (1.0 to 2.3)	.04
Invasive disease	1.0 (referent)	1.0 (0.6 to 1.5)	1.4 (0.9 to 2.1)	1.4 (0.9 to 2.2)	.08
<i>In situ</i> disease§	1.0 (referent)	1.6 (0.6 to 4.5)	1.9 (0.7 to 5.1)	2.3 (0.8 to 6.5)	.24
Testosterone, ng/dL					
MV RR‡	1.0 (referent)	0.9 (0.6 to 1.4)	1.5 (1.0 to 2.2)	1.6 (1.0 to 2.4)	<.001
Invasive disease	1.0 (referent)	0.7 (0.4 to 1.2)	1.4 (0.9 to 2.1)	1.4 (0.9 to 2.2)	.003
<i>In situ</i> disease§	1.0 (referent)	1.7 (0.5 to 5.5)	3.1 (1.0 to 9.3)	3.7 (1.2 to 11.0)	.01
Free testosterone, ng/dL					
MV RR‡	1.0 (referent)	1.4 (0.9 to 2.1)	1.6 (1.0 to 2.4)	1.7 (1.1 to 2.6)	<.001
Invasive disease	1.0 (referent)	1.3 (0.8 to 2.0)	1.5 (0.9 to 2.4)	1.8 (1.1 to 2.8)	<.001
<i>In situ</i> disease§	1.0 (referent)	2.4 (0.8 to 7.8)	3.9 (1.3 to 11.5)	1.7 (0.5 to 5.9)	.17
DHEA, ng/dL					
MV RR‡	1.0 (referent)	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	1.4 (0.9 to 2.2)	.02
Invasive disease	1.0 (referent)	1.2 (0.8 to 1.8)	1.1 (0.7 to 1.7)	1.3 (0.9 to 2.1)	.05
<i>In situ</i> disease§	1.0 (referent)	0.3 (0.1 to 1.2)	1.3 (0.5 to 3.1)	1.6 (0.7 to 3.8)	.22
DHEAS, µg/dL					
MV RR‡	1.0 (referent)	1.9 (1.2 to 2.9)	1.6 (1.1 to 2.5)	1.7 (1.1 to 2.7)	.003
Invasive disease	1.0 (referent)	2.1 (1.3 to 3.3)	1.7 (1.1 to 2.7)	1.8 (1.1 to 2.9)	.01
<i>In situ</i> disease§	1.0 (referent)	1.9 (0.7 to 5.1)	1.4 (0.5 to 3.9)	1.5 (0.5 to 4.0)	.26

*Batch-specific quartile cut points were used to categorize estradiol. The cut points for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cutpoints for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL. RR = relative risk; CI = confidence interval; MV = multivariable, PMH = postmenopausal hormones; SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate. Sample sizes were as follows: all women = 322 case subjects, 643 control subjects; invasive disease = 264; *in situ* disease = 41; never PMH use = 162; past PMH use = 160.

†P value, test for trend. The logarithm of the hormone level was entered into the model as a continuous variable; two-sided.

‡Conditional logistic regression models controlling for body mass index at age 18 years (<21, 21–22.9, 23–24.9, or ≥25 kg/m²), family history of breast cancer (yes or no), age at menarche (<12, 12, 13, or ≥14 y), age at first birth and parity (nulliparous; 1–4 children, first birth <25 y; 1–4 children, first birth 25–29 y; 1–4 children, first birth ≥30 y; ≥5 children, first birth <25 y; or ≥5 children, first birth ≥25 y), age at menopause (<46, 46–50, 51–55, or ≥56 y), and duration of PMH use (continuous) were used in the main analyses among all women. Unconditional logistic regression, controlling for the matching factors (age [5-year groups], month of blood draw [6-month blocks], time of blood draw [4-hour blocks], fasting status [<10 versus ≥10 hours]) and the same covariates as the conditional multivariable models were used for subgroup analyses.

§Unconditional logistic regression model controlling for matching factors only.

across fourths of DHEA and DHEAS, which were attenuated and no longer statistically significant (data not shown). The simple conditional models controlling for matching factors only differed negligibly (data not shown).

When analyses were restricted to those case subjects diagnosed from July 1, 1994, through June 30, 1998 [i.e., to the case subjects added since the publication of results from the 1990–1994 analysis (6)], the associations between hormone levels and breast cancer risk were similar or slightly stronger than those in the initial report. The greatest differences, when comparing the highest with lowest fourths in the 1990–1994 and 1994–1998 periods, respectively, were observed for estradiol (RR = 1.9, 95% CI = 1.1 to 3.5, and RR = 2.6, 95% CI = 1.5 to 4.7), testosterone (RR = 1.4, 95% CI = 0.7 to 2.7, and RR = 2.0, 95% CI = 1.1 to 3.6), and DHEA (RR = 1.1, 95% CI = 0.6 to 2.0, and RR = 2.0, 95% CI = 1.0 to 3.8). The only association to decrease slightly between analyses was that for DHEAS (for the 1990–1994 analysis, RR = 2.2, 95% CI = 1.1 to 4.2, and for the 1994–1998 analysis, RR = 1.5, 95% CI = 0.8 to 2.7). We observed negligible differences between the two follow-up periods for estrone (for the 1990–1994 analysis, RR = 2.0, 95% CI = 1.1 to 3.7, and for the 1994–1998 analysis, RR = 1.8, 95% CI = 1.0 to 3.4), estrone sulfate (for the 1990–1994 analysis, RR = 2.3, 95% CI = 1.2 to 4.1, and for the 1994–1998 analysis, RR = 2.5, 95% CI = 1.3 to 4.5), and androstenedione (for the 1990–1994 analysis, RR = 1.5, 95% CI = 0.8 to 2.8, and for the 1994–1998 analysis, RR = 1.7, 95% CI = 0.9 to 3.2).

Among estrogen metabolites, we observed a twofold increase in the risk of breast cancer associated with estradiol, free estradiol, estrone, or estrone sulfate, when the highest and lowest fourths were compared. The association with percent free estradiol was similar (RR = 1.4, 95% CI = 0.9 to 2.1), although we did not observe a statistically significant trend (P_{trend} = .11) (data not shown). When estradiol and testosterone were placed in the same multivariable model, the association with estradiol was essentially unchanged (RR = 1.9, comparing the highest with the lowest fourth of estradiol, 95% CI = 1.3 to 2.9; P_{trend} = .005), although the relative risk associated with testosterone was attenuated (RR = 1.2, comparing the highest with the lowest fourth of testosterone, 95% CI = 0.8 to 2.0; P_{trend} = .09) (data not shown). Associations of the estrogen metabolites with breast cancer risk were strongest among women who had never used postmenopausal hormones. However, statistically significant effect modification was observed for estrone and estradiol (P value, test for heterogeneity [$P_{\text{heterogeneity}}$] < .001 and $P_{\text{heterogeneity}}$ = .04, respectively), but associations of androgens with breast cancer risk did not vary statistically significantly when stratified by postmenopausal hormone use. We also observed that the relative risk was 50%–100% greater among case subjects with *in situ* disease than among case subjects with invasive disease for all hormones examined—except free estradiol, SHBG, free testosterone, DHEA, and DHEAS—although confidence intervals were wide and overlapped those of case subjects with invasive disease (Table 3). Polychotomous comparisons also were not statistically significant (data not shown). The associations observed among case subjects with *in situ* disease changed negligibly when case subjects with lobular (n = 3) or both lobular and intraductal (n = 4) tumors were excluded (data not shown).

We also evaluated potential effect modification of the association between endogenous hormone level and breast cancer risk by the following factors: age at blood collection (stratified at the control median = 63 years), age at cancer diagnosis (median = 67 years), time from menopause to blood collection (median = 13 years), waist-to-hip ratio (median = 0.79), and weight change from age 18 years to baseline (increase of <2 kg/m² compared with increase of 2 or more kg/m²). The majority of these interactions were not statistically significant. However, the association of androgen levels with the risk of breast cancer was statistically significantly stronger among women whose weight increased <2 kg/m² from age 18 years to baseline (comparing the highest fourth to the lowest fourth of androgen levels). Specifically, among these subjects, we observed statistically significant associations between breast cancer risk and the level of androstenedione (RR = 2.1, 95% CI = 1.2 to 3.8; $P_{\text{heterogeneity}}$ = .06), free testosterone (RR = 2.6, 95% CI = 1.4 to 4.7; $P_{\text{heterogeneity}}$ = .03), and DHEA (RR = 2.0, 95% CI = 1.2 to 3.6; $P_{\text{heterogeneity}}$ = .03) (data not shown).

When associations between hormone levels and the risk of breast cancer were evaluated according to receptor status of the tumor, the strongest associations and most consistent dose-response relations were observed among case subjects with ER⁺/PR⁺ tumors for all hormones except progesterone (Table 4). For example, among those with ER⁺/PR⁺ tumors, comparing the highest to lowest fourth of circulating hormone levels, we observed an increased risk associated with breast cancer for estradiol (RR = 3.3, 95% CI = 2.0 to 5.4; $P_{\text{heterogeneity}}$ < .001), for testosterone (RR = 2.0, 95% CI = 1.2 to 3.4; $P_{\text{heterogeneity}}$ = .009), for androstenedione (RR = 2.5, 95% CI = 1.4 to 4.3; $P_{\text{heterogeneity}}$ = .22), and for DHEAS (RR = 2.3, 95% CI = 1.3 to 4.1; $P_{\text{heterogeneity}}$ = .85). No linear trend was observed for any hormone among women with PR[−] tumors regardless of ER tumor status. We also investigated whether these tumor receptor-specific associations would differ when analyses were restricted to participants who had never used postmenopausal hormones. Although sample sizes of such subjects were very small, associations further restricted to case subjects with ER⁺/PR⁺ tumors tended to increase in magnitude. For example, among case subjects with ER⁺/PR⁺ tumors who had never used postmenopausal hormones, the risk of breast cancer associated with the top fourth of estradiol levels was approximately fivefold higher (RR = 4.8, 95% CI = 2.2 to 10.9) than that associated with the bottom fourth.

Finally, we evaluated the joint effect of estradiol fourths with fourths of progesterone and testosterone associated with the risk of breast cancer (Table 5). We found statistically significant Spearman correlations between estradiol and testosterone (r = .44) and between estrogen and progesterone (r = .15). However, tests for heterogeneity did not indicate statistically significant interactions between estradiol levels and either testosterone or progesterone levels. Regardless of testosterone level, the highest fourth of circulating estradiol concentration was associated with the greatest risk of breast cancer. Although this pattern was also true for the stratification of estradiol by progesterone level, there was an indication that high levels of endogenous progesterone among women with the lowest amount of circulating estradiol were associated with a decreased risk for breast cancer (for the comparison of the lowest fourth of estradiol but the top half of progesterone levels to those with the lowest fourths of both hormones, RR

Table 4. Risk of breast cancer according to fourths of plasma hormone levels by tumor receptor status*

Plasma hormone (No. case subjects/ No. control subjects)	RR (95% CI)				<i>P</i> _{trend} †	<i>P</i> _{heterogeneity} ‡
	1	2	3	4		
Estradiol						<.001
ER ⁺ /PR ⁺ (153/637)	1.0 (referent)	1.8 (1.0 to 3.0)	1.5 (0.8 to 2.8)	3.3 (2.0 to 5.4)	<.001	
ER ⁺ /PR ⁻ (38/637)	1.0 (referent)	1.0 (0.4 to 2.2)	0.5 (0.2 to 1.7)	1.0 (0.4 to 2.4)	.46	
ER ⁻ /PR ⁻ (33/637)	1.0 (referent)	0.8 (0.3 to 2.1)	0.8 (0.3 to 2.4)	1.0 (0.4 to 2.6)	.82	
Estrone						.04
ER ⁺ /PR ⁺ (153/624)	1.0 (referent)	1.2 (0.7 to 2.1)	1.6 (0.9 to 2.8)	2.4 (1.4 to 4.1)	<.001	
ER ⁺ /PR ⁻ (38/624)	1.0 (referent)	0.5 (0.2 to 1.3)	1.0 (0.4 to 2.4)	0.7 (0.3 to 1.7)	.92	
ER ⁻ /PR ⁻ (34/624)	1.0 (referent)	0.6 (0.2 to 1.7)	0.6 (0.2 to 1.6)	0.9 (0.4 to 2.3)	.92	
Estrone sulfate						.80
ER ⁺ /PR ⁺ (150/622)	1.0 (referent)	1.5 (0.8 to 2.7)	1.6 (0.9 to 3.0)	2.8 (1.6 to 4.9)	<.001	
ER ⁺ /PR ⁻ (39/622)	1.0 (referent)	1.5 (0.6 to 4.0)	0.9 (0.3 to 2.7)	1.9 (0.7 to 4.8)	.34	
ER ⁻ /PR ⁻ (32/622)	1.0 (referent)	0.8 (0.3 to 2.3)	0.6 (0.2 to 1.8)	1.3 (0.5 to 3.4)	.28	
Progesterone						.22
ER ⁺ /PR ⁺ (131/530)	1.0 (referent)	1.3 (0.7 to 2.3)	1.3 (0.8 to 2.2)	1.3 (0.7 to 2.4)	.38	
ER ⁺ /PR ⁻ (34/530)	1.0 (referent)	0.9 (0.3 to 2.4)	1.1 (0.5 to 2.6)	0.3 (0.1 to 1.3)	.17	
ER ⁻ /PR ⁻ (28/530)	1.0 (referent)	0.8 (0.2 to 2.5)	0.8 (0.3 to 2.3)	0.8 (0.3 to 2.7)	.82	
SHBG						.002
ER ⁺ /PR ⁺ (147/622)	1.0 (referent)	0.7 (0.4 to 1.1)	0.6 (0.4 to 1.0)	0.5 (0.3 to 0.8)	.001	
ER ⁺ /PR ⁻ (38/622)	1.0 (referent)	0.4 (0.2 to 1.3)	0.7 (0.3 to 1.9)	1.1 (0.5 to 2.8)	.78	
ER ⁻ /PR ⁻ (33/622)	1.0 (referent)	1.6 (0.5 to 4.6)	0.7 (0.2 to 2.7)	1.8 (0.6 to 5.0)	.72	
Testosterone						.03
ER ⁺ /PR ⁺ (149/628)	1.0 (referent)	0.9 (0.5 to 1.7)	1.8 (1.1 to 3.1)	2.0 (1.2 to 3.4)	<.001	
ER ⁺ /PR ⁻ (38/628)	1.0 (referent)	0.4 (0.2 to 1.1)	0.6 (0.3 to 1.6)	0.7 (0.3 to 1.6)	.35	
ER ⁻ /PR ⁻ (33/628)	1.0 (referent)	0.9 (0.3 to 2.9)	1.4 (0.5 to 3.9)	1.9 (0.7 to 5.0)	.12	
Androstenedione						.22
ER ⁺ /PR ⁺ (148/621)	1.0 (referent)	1.5 (0.9 to 2.7)	1.9 (1.1 to 3.3)	2.5 (1.4 to 4.3)	<.001	
ER ⁺ /PR ⁻ (38/621)	1.0 (referent)	0.7 (0.3 to 1.9)	0.9 (0.4 to 2.4)	0.9 (0.4 to 2.4)	.73	
ER ⁻ /PR ⁻ (34/621)	1.0 (referent)	0.5 (0.2 to 1.6)	1.1 (0.4 to 2.6)	0.7 (0.3 to 2.0)	.43	
DHEA						.76
ER ⁺ /PR ⁺ (145/603)	1.0 (referent)	1.3 (0.8 to 2.2)	1.1 (0.6 to 1.9)	1.6 (0.9 to 2.7)	.05	
ER ⁺ /PR ⁻ (36/603)	1.0 (referent)	1.3 (0.4 to 3.5)	1.7 (0.6 to 4.7)	1.5 (0.5 to 4.2)	.26	
ER ⁻ /PR ⁻ (32/603)	1.0 (referent)	0.9 (0.3 to 2.8)	1.2 (0.4 to 3.2)	1.0 (0.3 to 2.9)	.77	
DHEAS						.85
ER ⁺ /PR ⁺ (153/634)	1.0 (referent)	2.4 (1.3 to 4.1)	1.9 (1.1 to 3.5)	2.3 (1.3 to 4.1)	.002	
ER ⁺ /PR ⁻ (38/634)	1.0 (referent)	0.9 (0.3 to 2.5)	1.0 (0.4 to 2.6)	1.4 (0.5 to 3.5)	.24	
ER ⁻ /PR ⁻ (33/634)	1.0 (referent)	0.5 (0.2 to 1.5)	0.7 (0.2 to 1.8)	1.1 (0.4 to 2.7)	.94	

*Unconditional logistic regression model controlling for the following matching factors only: age (5-year groups), month of blood collection (6-month blocks), time of blood collection (4-hour blocks), fasting status (<10 versus ≥10 hours). RR = relative risk; CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor; SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate.

†*P* value, test for trend. The logarithm of the hormone level was entered into the model as a continuous variable; two-sided.

‡*P* value, test for heterogeneity. Likelihood ratio test calculated from polychotomous logistic regression; two-sided, two degrees of freedom.

= 0.5, 95% CI = 0.2 to 1.3). When these analyses were restricted to case subjects with ER⁺/PR⁺ tumors, similar patterns were observed.

DISCUSSION

Among the 322 case subjects and 643 control subjects included in this nested case-control analysis, we observed a statistically significant direct association between the endogenous levels of each steroid hormone evaluated and the risk of breast cancer, with the exceptions of the endogenous levels of progesterone and SHBG. We observed the greatest magnitudes of effect among case subjects with ER⁺/PR⁺ tumors. Strengths of this study include its size; prospectively collected environmental, reproductive, and biomarker data that reduced concerns of recall bias or blood sample timing relative to breast cancer diagnosis; and the collection of detailed tumor-specific data.

The relation between endogenous steroid hormones and breast cancer risk has been evaluated in nine prospective

epidemiologic studies (6,21–31), including our own with follow-up from the 1990–1994 analysis (6). Recently, data from these studies were pooled and re-analyzed (1). For all hormones evaluated, our results are consistent with those of this collaborative study (1). Similarly, the results that we observed for 1990–1994 and 1994–1998 analyses were similar, suggesting that a single blood sample can predict breast cancer risk for at least 8 years after collection. This result is also consistent with the results observed by the NYU Women's Health Study, which has recently added 7 years of follow-up to their original analysis (32). We observed stronger associations among women who had never used postmenopausal hormones because a single blood sample likely best reflects long-term hormone levels in these women. That the relations with breast cancer risk among women who had never used postmenopausal hormones are most apparent for estrone, estradiol, and estrone sulfate was expected, because these hormones are most affected by Premarin, the predominant postmenopausal hormone used in this population.

Table 5. Estradiol, testosterone, and progesterone in relation to breast cancer risk presented as relative risks (RRs) and 95% confidence intervals (CIs)*

	Estradiol fourths			
	1†	2†	3†	4†
Testosterone fourths				
<15 ng/dL	1.0 (referent)	1.0 (0.5 to 2.1)	1.1 (0.4 to 3.3)	3.8 (1.5 to 9.9)
15–19 ng/dL	1.0 (0.5 to 1.9)	0.9 (0.4 to 1.9)	0.9 (0.4 to 2.2)	1.5 (0.7 to 3.5)
20–26 ng/dL	1.5 (0.7 to 3.0)	2.1 (1.1 to 4.0)	1.5 (0.7 to 2.9)	2.2 (1.2 to 4.1)
>26 ng/dL	1.1 (0.4 to 2.7)	2.0 (1.0 to 3.9)	1.3 (0.6 to 2.8)	2.4 (1.4 to 4.2)
				<i>P</i> _{heterogeneity} = .33
Progesterone fourths				
<1.6 ng/dL	1.0 (referent)	0.7 (0.4 to 1.4)	0.6 (0.3 to 1.5)	1.7 (0.9 to 3.3)
1.6–4 ng/dL	1.2 (0.6 to 2.5)	0.6 (0.2 to 1.5)	1.2 (0.5 to 3.1)	1.4 (0.7 to 3.1)
4.1–8 ng/dL	0.5 (0.2 to 1.1)	1.6 (0.8 to 3.2)	1.0 (0.5 to 2.1)	1.8 (0.9 to 3.7)
>8 ng/dL	0.5 (0.2 to 1.3)	1.1 (0.5 to 2.4)	0.8 (0.3 to 2.2)	1.4 (0.7 to 2.9)
				<i>P</i> _{heterogeneity} = .50

*Unconditional logistic regression model controlling for the following matching factors only: age (5-year groups), month of blood collection (6-month blocks), time of blood collection (4-hour blocks), fasting status (<10 versus ≥10 hours).

†Batch-specific quartile cut points were used to categorize estradiol. Cut points 1–4, respectively, for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cut points 1–4, respectively, for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL.

We are, to our knowledge, the first to report analyses that distinguish between endogenous levels of steroid hormones and the risk for invasive and *in situ* disease. We observed modest differences between these associations, with the associations with *in situ* cancer being generally of greater magnitude. These findings are consistent with the 50% or greater reduction in *in situ* breast cancer with tamoxifen use (33). It has been argued that the increased risk of *in situ* disease among postmenopausal hormone users may be a diagnostic bias reflecting more frequent and detailed examination of women exposed to exogenous hormones (34–36). However, our data suggest that the association is biologic, at least in part. Exclusion of case subjects with lobular disease did not measurably alter the associations that we observed; however, further study in larger case populations are needed.

In addition, our findings suggest that higher concentrations of endogenous steroids—both estrogens and androgens—are primarily associated with an increased risk of ER⁺/PR⁺ breast cancers. We have recently reported that both body mass index and current postmenopausal hormone use are preferentially associated with an increased risk of ER⁺/PR⁺ tumors in this cohort (37). However, in the only prior assessment of endogenous estrogens by tumor ER status (38), no differences between the effect of estrogens on the risk of ER⁺ versus PR[−] tumors were observed, although only 53 case subjects with ER⁺ tumors and 23 case subjects with ER[−] tumors were evaluated.

The association with an increased risk for ER⁺/PR⁺ tumors and higher levels of endogenous steroid hormones is biologically feasible because the presence of ERs and PRs in cancer cells is considered to provide a growth advantage, as shown by the positive association between the phenotype and high proliferative activity (39). ER overexpression has been associated with mammary tumors in animal models, and selective estrogen receptor modulators, such as tamoxifen, block ER activation in the breast, suggesting that ER-mediated regulation of gene expression plays a biologically important role in normal and malignant cells (39–41). In addition, chemoprevention trials evaluating selective estrogen receptor modulators have found a decreased incidence of ER⁺ tumors associated with use of such modulators (33,42–44), and it has been suggested that efficacy of such drugs may differ by the woman's underlying hormone profile (45). Less is known about the influence of chemopreventive agents or

agonists on PRs, although the level PR action seems to be dependent upon ER action and thus is an indication of a functional ER (46).

To our knowledge, our study was the first to investigate whether progesterone levels are associated with breast cancer risk in postmenopausal women, and we observed no statistically significant association. Interestingly, we observed that case subjects with PR⁺ tumors were statistically significantly most strongly affected by all circulating steroid hormones, except for progesterone. On the basis of largely indirect evidence, progesterone has been hypothesized to decrease breast cancer risk by opposing estrogenic stimulation of the breast (47,48) and to increase risk because breast mitotic rates are highest in the luteal (high progesterone) phase of the menstrual cycle (49–51). Results of murine studies suggest that implantation of progesterone inhibits apoptosis in the mammary gland (52) and that the progesterone signal contributes to mammary tumor susceptibility (53). It is possible that the range of progesterone concentrations among subjects in our study was not wide enough to detect a trend when comparing the highest with the lowest fourths or that the typical level of circulating progesterone among postmenopausal women is indeed too low to initiate or promote breast neoplasia. Results from epidemiologic studies of the association between endogenous progesterone and breast cancer risk in premenopausal women have been inconsistent, with non-statistically significant positive (54,55) and inverse (23,56) associations being reported.

Studies of postmenopausal hormone use have consistently shown that a greater risk of breast cancer is associated with the use of formulations containing estrogen and progesterone than with the use of formulations containing only estrogen (2–5,51). However, even when stratified by fourths of endogenous estradiol concentration, we did not observe such an interaction between progesterone and estradiol levels. Given the lack of association that we observed with naturally circulating progesterone and the relatively strong associations observed with synthetic progestin exposure (2–5), it may be that synthetic progestins have a more dramatic or metabolically different effect on breast tissue proliferation. In recent studies conducted in breast cancer cell lines, the type, dose, and regimen of progestogen used influenced growth stimula-

tion (57–61) and the pro- or antiapoptotic effect observed (62,63).

Overall, our data confirm the important role for circulating steroid hormones in the etiology of breast cancer. We also observed that the history of postmenopausal hormone use and the receptor status of a breast tumor may modify these relations. Although we did not observe a direct association between the risk of breast cancer and progesterone levels, additional studies of this association are warranted. A key question is whether endogenous hormone levels could add substantially to the ability to predict an individual woman's risk of breast cancer beyond standard breast cancer risk factors—particularly, body mass index—to identify those who would most benefit from increased screening or chemoprevention [e.g., with tamoxifen (42), raloxifene (43), or aromatase inhibitors (64)]. Additional tumor subtype-specific analyses may further elucidate the underlying mechanisms of these relations and lead to more targeted and efficacious hormone-based prevention protocols.

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NOTES

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PRACTICE OF EPIDEMIOLOGY

Adult Recall of Adolescent Diet: Reproducibility and Comparison with Maternal Reporting

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Many cancers have long latency periods, and dietary factors in adolescence may plausibly affect cancer occurrence in adulthood. Because of a lack of prospective data, retrospective collection of data on adolescent diet is essential. The authors evaluated a 124-item high school food frequency questionnaire (HS-FFQ) assessing diet during high school (15–35 years in the past) that was completed in 1998 by 45,947 US women in the Nurses' Health Study II (NHSII) cohort. To assess reproducibility, the authors readministered the HS-FFQ approximately 4 years later to 333 of these women. The mean Pearson correlation for 38 nutrient intakes was 0.65 (range, 0.50–0.77), and the mean Spearman rank correlation for food intakes was 0.60 (range, 0.37–0.77). Current adult diet was only weakly correlated with recalled adolescent diet (for nutrient intakes, mean $r = 0.20$). For assessment of validity, 272 mothers of the NHSII participants were asked to report information on their daughters' adolescent diets using the HS-FFQ. In this comparison, the mean Pearson correlation was 0.40 (range, 0.13–0.59) for nutrients, and the mean Spearman rank correlation for foods was 0.30 (range, 0.10–0.61). While further studies are warranted, these findings imply that this food frequency questionnaire provides a reasonable record of adolescent diet.

adolescent; diet; mental recall; mothers; nutrition; questionnaires; reproducibility of results

Abbreviations: HS-FFQ, high school food frequency questionnaire; NHSII, Nurses' Health Study II.

Many common cancers have long latency periods that may span several decades between the onset of the carcinogenic process and clinical detection (1). Dietary factors in adolescence may plausibly affect cancer occurrence in adulthood by enhancing or deterring carcinogenic processes (2). Adolescence is characterized by hormonal changes and rapid proliferation of incompletely differentiated tissues in several organs. Thus, adolescence may be a more etiologically relevant period than adulthood for the study of potential causal

and preventive determinants of some cancers (3–5). A better understanding of which dietary factors are important in the etiology of cancer and the period of life in which they act is critical.

Although prospective studies assessing food intake among children and adolescents have begun, most will require many more decades of follow-up to reach clinical endpoints (1). A more timely though potentially less ideal way of assessing the relation between adolescent diet and cancer is collection

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of data retrospectively from adults. If these dietary data are collected before disease occurrence, recall bias is avoided.

A crucial component in the conduct and interpretation of studies using retrospective dietary assessment is evaluation of the questionnaire instrument. Recall of adolescent diet as an adult will be prone to measurement error, because it relies primarily on memory of diet in the distant past. Several studies have reported reasonable validity and reproducibility of data on recalled diet up to 10 years in the past (6–9). However, greater uncertainty exists for recall exceeding 10 years (10, 11).

We evaluated a food frequency questionnaire that asked women in the Nurses' Health Study II (NHSII) cohort about foods they had eaten in high school, between the ages of 13 and 18 years. In this paper, we report on 1) the reproducibility of this questionnaire, using recalled information on adolescent diet provided by participants at two different time points; 2) a maternal comparison in which these recalled data were compared with information on high school diet provided by mothers of NHSII participants; and 3) the influence of current adult diet on recall of adolescent diet by NHSII participants.

MATERIALS AND METHODS

Study participants

The NHSII is an ongoing prospective study of a cohort of 116,671 US female registered nurses. When the study was initiated in 1989, participants were between the ages of 25 and 42 years. Every 2 years, participants have been sent a follow-up questionnaire asking about the use of hormones, lifestyle practices, and diagnoses of chronic disease. Every 4 years, participants also receive a semiquantitative food frequency questionnaire with which to report their current diet. The study has maintained a response rate of 90 percent or greater (12).

The high school food frequency questionnaire (HS-FFQ), a supplementary questionnaire administered in 1998, was completed by 45,947 NHSII women. For assessment of reproducibility, 400 women were randomly selected from these initial participants to complete a second HS-FFQ in 2002. To minimize recall bias due to existing disease, participants who had cancer, heart disease, or asthma were excluded from the second sample. This second HS-FFQ was completed by 347 (87 percent) of 400 women. Fourteen women were subsequently excluded on the basis of established dietary criteria (caloric intake <600 kcal/day or >5,000 kcal/day, more than 70 food items left blank, or more than one food section left blank, other than dairy or meat sections), leaving a total of 333 women for the reproducibility analysis.

Maternal reports of NHSII adolescent diet were obtained from participants in the Nurses' Mothers Cohort Study. This study was begun in 2001 to investigate the effects of perinatal and early-life exposures on adult disease, and it includes 35,830 mothers of NHSII participants. To select participants for the comparison of recalled high school diets between the NHSII women and their mothers, we randomly selected 400 NHSII participants who completed the initial

HS-FFQ and whose mothers were respondents in the Nurses' Mothers Cohort Study. Those NHSII women who had cancer, heart disease, or asthma were excluded. We also excluded participants who were selected for the reproducibility substudy in order to reduce respondent burden. In addition, to obtain the best possible independent comparison of responses to the HS-FFQ, we included only mothers who were early respondents in the Nurses' Mothers Cohort Study and who said they had completed that questionnaire without the help of their daughters.

Among the 400 selected NHSII participants, 358 (90 percent) gave permission and provided current address information with which to contact their mothers. These mothers were then sent an HS-FFQ with instructions not to discuss their responses with their daughters before returning it. Of the 358 contactable mothers, 302 (84 percent) completed the questionnaire. Six mothers were excluded from the analysis on the basis of the established dietary exclusion criteria (described above). Another 24 mothers were excluded because they skipped two or more consecutive questionnaire pages. Thus, a total of 272 mothers were analyzed.

This study was approved by the Partners Institutional Review Board at Brigham and Women's Hospital (Boston, Massachusetts).

HS-FFQ

The HS-FFQ is a 124-item, self-administered food frequency questionnaire (available online) (13). Questions posed to NHSII participants included how often, on average, they had consumed a specified food, beverage, or vitamin (described hereafter as "foods") when they were between the ages of 13 and 18 years, or approximately high school age. This food frequency questionnaire was modeled on other validated questionnaires administered in the Nurses' Health Study and NHSII cohorts (11, 14, 15). Foods included were those commonly consumed by American adults during the years when the participants were in high school (1960–1982), as assessed in earlier investigations (15). Foods of interest to cancer researchers, such as major contributors of fat, fiber, and antioxidant vitamins, were included. We took secular changes in food formulation into account by using an NHSII participant's year of birth to assign different nutrient profiles for specific foods. Serving sizes were listed in natural units whenever possible (e.g., one apple, one glass of milk, or one slice of bread) and otherwise were based on the most common portion size reported in the US Department of Agriculture's Nationwide Food Consumption Survey (1977–1978) (16). The response choices for food items consisted of nine possible frequencies, ranging from "almost never" to "six or more times per day." Questions about the use of multivitamin supplements and vitamin C supplements had five possible response choices, ranging from a frequency of zero to 10 or more per week.

Nutrient intakes for each individual were calculated by multiplying the nutrient content of each food and supplement by the frequency of consumption relative to once per day and then summing the contribution from all foods and supplements (described hereafter as "nutrients" for convenience, recognizing that constituents such as caffeine are not nutritive

components). The database for the nutrient analysis was constructed primarily from information provided by US Department of Agriculture handbooks and bulletins for foods consumed during the period when NHSII participants were in high school (17–19).

Analysis

We adjusted nutrient data for energy intake using the residual method described by Willett and Stampfer (20), to account for variation in nutrient intakes due to total energy intake. We calculated mean values and standard deviations to characterize intakes and between-person variation in nutrient and food intakes. We transformed nutrient data by natural logarithm to improve their normality for the correlation analyses (11).

To examine reproducibility, we calculated intraclass correlations for nutrients and Spearman rank correlations for foods from the two HS-FFQs completed by the NHSII women.

In addition, we evaluated the potential for confounding of reported high school diet by current diet by calculating Pearson correlations between NHSII participants' nutrient intakes in the first HS-FFQ and their current nutrient intakes in 1995 (the last adult diet measurement prior to the 1998 HS-FFQ).

We also assessed the influence of misreporting of dietary intake on the reproducibility correlations. To identify underreporting, we used the Goldberg cutoff for the ratio of energy intake to basal metabolic rate or physical activity level (21, 22). The calculation of this cutoff has been reviewed by Black (21) and Goldberg et al. (22). We chose a physical activity level of 1.73 based on doubly labeled water energy expenditure data for adolescent girls and then calculated the lower confidence limit (cutoff) for it using values cited by Black et al. (21, 22). To further assess misreporting, we used the sex- and age-specific equations developed by the World Health Organization to calculate the ratio of reported intake to the predicted energy expenditure for NHSII participants when they were adolescents (23). To do this, we calculated the basal metabolic rate for each participant on the basis of her self-reported weight at age 18 years. This value was then multiplied by a physical activity level of 1.5 on the basis of data from the World Health Organization, assuming 2.5 hours of daily moderate physical activity (23). We next calculated the ratio of reported energy intake (using reported calories from the first administration of the HS-FFQ) to this predicted energy expenditure for each individual. Using these ratios, we classified women as "underreporters" (ratio values in the lowest 20 percent of the distribution) or "high reporters" (ratio values in the top 20 percent of the distribution) and the remaining women as "acceptable reporters" for total energy intake and compared the reproducibility correlations between these three groups. Since physical activity level was the same for all participants, the percentage cutpoints for this second method depended on the value for the ratio of energy intake to the basal metabolic rate.

To evaluate the comparability of adolescent diets reported by NHSII participants and their mothers (maternal comparison), we calculated Pearson correlations for nutrients and

Spearman rank correlations for foods. We also used Pearson correlations to assess associations between NHSII participants' current diets and their mothers' recall of their adolescent diets.

RESULTS

Reproducibility

The mean age of the NHSII participants at the first administration of the HS-FFQ was 43.8 years (range, 33.6–53.3); thus, diet recall exceeded an average of 25 years in the past. The mean age of the subsample at the administration of the second HS-FFQ approximately 4 years later was 48.9 years (range, 38.9–56.4). The women in both the first administration of the HS-FFQ and the second administration were similar with regard to several demographic variables (table 1) and were also similar to the entire NHSII cohort, from which they were originally sampled.

The nutrient correlations between the first and second NHSII participant recalls were moderate to good, with an average correlation of 0.65 and a range of 0.50–0.77 (table 2). Highly reproducible nutrient values included total vitamin C ($r = 0.77$), total vitamin B₂ ($r = 0.76$), and caffeine ($r = 0.74$). The nutrients measured with the least precision were alcohol ($r = 0.50$) and vitamin B₁₂, both total ($r = 0.52$) and without supplements ($r = 0.51$).

The correlations between nutrient intakes calculated from the 1995 current diet and those calculated from the first recall of high school diet were low, with an average correlation of 0.20 and a range of –0.11 to 0.43 (table 2). Moreover, the correlations remained low when we used current diet as reported in 1999, 1 year after the first high school recall was administered (mean $r = 0.20$; range, 0.01–0.44).

In our analysis of misreporting, we found no appreciable underreporting on either the group level or the individual level using the Goldberg cutoff (21, 22). Our calculated lower limit (the Goldberg cutoff for underreporting) for the ratio of reported energy intake to predicted basal metabolic rate was 1.70 for the overall group. Our study's group mean of 1.9 was higher than this cutoff, suggesting that the reported energy intakes of NHSII participants were reasonable in relation to underreporting. On the individual level, our calculated lower limit ratio value was 1.26, and only 9.0 percent of NHSII participants were below this lower limit. After exclusion of this 9.0 percent, our findings did not change appreciably ($r = 0.64$) for nutrients. When we used another method based on values cited by the World Health Organization (23), the average nutrient correlations were similar for "underreporters" ($r = 0.63$; 20 percent prevalence), "high reporters" ($r = 0.66$; 20 percent prevalence), and "acceptable reporters" ($r = 0.64$; 60 percent prevalence).

To examine reproducibility further, we jointly classified nutrient intakes from the two administrations of the HS-FFQ into quintiles and calculated the percentage of responses plus or minus one quintile. Eighty percent of the nutrient values from the second administration of the HS-FFQ were within one quintile of values from the first administration.

The correlations for foods were slightly lower than those for nutrients, with an average of 0.60 and a range of 0.37–

TABLE 1. Characteristics of all Nurses' Health Study II participants who responded to a high school food frequency questionnaire in 1998 as compared with those selected in 2002 for reproducibility and maternal comparison substudies

	All HS-FFQ* respondents (n = 45,947)	Reproducibility substudy (n = 333)	Maternal comparison substudy (n = 272)
Mean age (years)	44	49	47
Mean body mass index† at age 18 years, at baseline‡	21	21	21
Mean body mass index§	26	26	25
Mean age (years) at first birth§	26	26	27
Premenopausal (%)§	84	84	92
Nulliparous (%)§	7	9	9
Current smoker (%)§	9	6	7

* HS-FFQ, high school food frequency questionnaire.

† Weight (kg)/height (m)².

‡ Value reported in 1989.

§ Value reported in 1997.

0.77. Foods with highly reproducible values included iced tea ($r = 0.77$), diet soda with caffeine ($r = 0.76$), and milk ($r = 0.76$). Foods with the lowest reproducibility were diet soda without caffeine ($r = 0.37$), onion eaten as a vegetable ($r = 0.42$), and raw spinach ($r = 0.42$). Individual correlations for all foods are available online (24). When intakes were grouped into food categories, the mean correlations between the first and second administrations were good: for dairy foods, $r = 0.64$; for (nondairy) beverages, $r = 0.70$; for main dishes, $r = 0.57$; for bread/cereals/grains, $r = 0.48$; for fruit, $r = 0.67$; and for vegetables, $r = 0.64$. Red meat consumed within main dishes had a mean correlation of 0.52.

Comparison with maternal reports

The mean age of the mothers who responded was 73 years (range, 58–89 years). The NHSII participants represented by the mothers were similar in terms of several demographic variables to the 45,947 respondents in the first high school diet recall (table 1) and also similar to the entire NHSII cohort.

The nutrient correlations between the NHSII participants' recalls and their mothers' recalls were moderate, with a mean of 0.40 and a range of 0.13–0.59 (table 3). Nutrients with the highest correlations were animal fat and vegetable fat: Both had a correlation of 0.51. Nutrients with the lowest correlations were total calories ($r = 0.13$), retinol ($r = 0.30$), and monounsaturated fat ($r = 0.30$). NHSII participants' current nutrient intakes, as assessed in 1995, were only weakly correlated with their mothers' recall of their high school diets (mean nutrient correlation: $r = 0.13$).

Overall, the correlations comparing mothers' reports with their daughters' reports were lower for foods than for nutrients, with a mean of 0.30 and a range of 0.10–0.61 for foods. The foods with the highest correlations were iced tea ($r = 0.61$) and orange juice ($r = 0.52$). Those with the lowest correlations were brownies ($r = 0.10$) and soda without

caffeine ($r = 0.10$). Individual correlations for all foods are available online (24).

DISCUSSION

In this study, we evaluated the reproducibility of a food frequency questionnaire that asked adult participants, at an interval of 4 years, about their diet in high school, 15–35 years in the past. We also compared participants' recalls with information on high school diet provided by their mothers. The mothers' reports were intended as independent estimates of their daughters' high school diets and thus a measure, though not an ideal one, of validity.

Reproducibility

Our results indicate moderate-to-good reproducibility for foods and nutrients and appear to be consistent with the handful of studies to date that have examined remotely recalled adolescent diet. Previously, we examined the reproducibility of a shorter 24-item adolescent diet questionnaire administered twice at an interval of 2 years to participants in the Nurses' Health Study, a cohort that is similar to but older than the women in the NHSII (12, 14). We reported average correlations of 0.57 for 24 foods (range: from 0.38 for beef to 0.73 for orange juice) and 0.48 for nutrients (range: from 0.34 for vitamin E to 0.68 for cholesterol). Wolk et al. (25) examined the short-term reliability (9–12 months) of adolescent diet recalled over 20 years later by healthy controls in a Swedish case-control study; they reported a correlation of 0.46 for both foods and nutrients for a 45-item food frequency questionnaire.

The influence of current diet is an important possible source of bias for the assessment of remote diet. For instance, our reproducibility results could potentially be overestimated if NHSII participants simply reported their current diet at both administrations of the questionnaire. However, the low correlations between current diet and

TABLE 2. Correlations for daily nutrient intake between two recalls of high school diet and between recalled high school diet and current diet, Nurses' Health Study II (reproducibility substudy)

Daily nutrient intake*	Mean reported value			Intraclass correlation between first HS-FFQ and second HS-FFQ†,‡	Pearson correlation between first HS-FFQ and current adult diet†
	First HS-FFQ (1998) (n = 333)	Second HS-FFQ (2002) (n = 333)	Current adult diet (1995) (n = 308)§		
Total calories (kcal)	2,766	2,669	1,752	0.69	0.43
Total fat (g)	124	123	57	0.62	0.20
Animal fat (g)	79	79	33	0.66	0.21
Vegetable fat (g)	44.8	45	24	0.64	0.16
Saturated fat (g)	49	49	20	0.66	0.24
Monounsaturated fat (g)	44	44	22	0.60	0.23
Polyunsaturated fat (g)	20	20	10	0.58	0.07
Trans fat (g)	7	7	3	0.62	0.19
Cholesterol (mg)	439	434	225	0.59	0.18
Protein (g)	106	106	86	0.57	0.23
Carbohydrates (g)	312	313	240	0.58	0.19
Glycemic index¶, bread	78	78	77	0.60	0.29
Glycemic load#, bread	245	245	183	0.58	0.24
Total fructose (g)	70	70	43	0.65	0.17
Dietary fiber (g)	21	21	20	0.67	0.38
Vitamin A (RE‡ (µg))	1,860	1,865	2,159	0.71	0.29
Without supplements (RE (µg))	1,679	1,718	1,515	0.66	0.32
Retinol (µg)	929	880	1,033	0.63	0.16
Without supplements (µg)	746	733	518	0.52	0.06
Beta-carotene (µg)	3,810	3,969	5,293	0.72	0.32
Vitamin E (mg)	13	13	47	0.62	-0.11
Without supplements (mg)	13	13	8	0.61	0.14
Vitamin C (mg)	164	164	326	0.77	0.28
Without supplements (mg)	143	145	142	0.72	0.43
Riboflavin (vitamin B ₂) (mg)	2	2	4	0.76	0.17
Without supplements (mg)	2	2	2	0.72	0.18
Pyridoxine (vitamin B ₆) (mg)	2	2	10	0.73	0.07
Without supplements (mg)	2	2	2	0.64	0.25
Vitamin B ₁₂ (µg)	9	8	10	0.52	0.08
Without supplements (µg)	8	8	6	0.51	0.15
Vitamin D (µg)	9	9	10	0.71	0.16
Without supplements (µg)	8	8	6	0.68	0.22
Total folate (µg)	327	328	485	0.72	0.20
Without supplements (µg)	317	321	321	0.67	0.33
Calcium (mg)	1,081	1,101	1,036	0.73	0.13
Iron (mg)	14	14	25	0.61	-0.02
Caffeine (mg)	91	82	210	0.74	0.22
Alcohol (g)	0.2	0.3	3	0.50	0.14
Average				0.65	0.20

* All nutrient data were adjusted for energy intake; nutrient data for the correlations were also log-transformed.

† All *p* values were two-sided, and all correlations larger than 0.11 were significant at the 0.05 level.

‡ HS-FFQ, high school food frequency questionnaire; RE, retinol equivalents.

§ Only 308 women who had responded to the 1995 Nurses' Health Study II (current) adult diet questionnaire had also completed the 1998 HS-FFQ.

¶ Postprandial rise in serum glucose level compared with the rise in serum glucose for 1 g of reference carbohydrate (white bread).

Each unit of dietary glycemic load represents the glycemic equivalent of 1 g of carbohydrate from white bread.

TABLE 3. Correlations for daily nutrient intake in high school as reported by adult women and their mothers, Nurses' Health Study II (maternal comparison substudy)

Daily nutrient intake*	Mean reported value		Pearson correlation between first HS-FFQ and mothers' HS-FFQ†,‡
	First HS-FFQ (1998) (n = 272)	Mothers' HS-FFQ (2002) (n = 272)	
Total calories (kcal)	2,807	2,289	0.13
Total fat (g)	124	99	0.32
Animal fat (g)	77	61	0.51
Vegetable fat (g)	47	37	0.51
Saturated fat (g)	48	39	0.47
Monounsaturated fat (g)	44	35	0.30
Polyunsaturated fat (g)	21	16	0.35
Trans fat (g)	7	6	0.45
Cholesterol (mg)	426	348	0.34
Protein (g)	107	84	0.42
Carbohydrates (g)	312	253	0.33
Glycemic index§, bread	78	77	0.43
Glycemic load¶, bread	245	196	0.38
Total fructose (g)	70	58	0.31
Dietary fiber (g)	20	17	0.35
Vitamin A (RE‡ (µg))	1,801	1,710	0.42
Without supplements (RE (µg))	1,644	1,426	0.42
Retinol (µg)	846	879	0.30
Without supplements (µg)	688	595	0.32
Beta-carotene (µg)	3,833	3,307	0.33
Vitamin E (mg)	13	10	0.38
Without supplements (mg)	13	10	0.36
Vitamin C (mg)	160	153	0.43
Without supplements (mg)	137	125	0.39
Riboflavin (vitamin B ₂) (mg)	2	2	0.42
Without supplements (mg)	2	2	0.59
Pyridoxine (vitamin B ₆) (mg)	2	2	0.41
Without supplements (mg)	2	2	0.43
Vitamin B ₁₂ (µg)	8	7	0.43
Without supplements (µg)	8	7	0.42
Vitamin D (µg)	9	9	0.48
Without supplements (µg)	8	7	0.46
Total folate (µg)	322	276	0.47
Without supplements (µg)	315	264	0.49
Calcium (mg)	1,103	893	0.47
Iron (mg)	15	11	0.47
Caffeine (mg)	83	51	0.47
Average			0.40

* All nutrient data were adjusted for energy intake; nutrient data for the correlations were also log-transformed.

† All *p* values for Pearson correlations were two-sided and were significant at the 0.05 level.

‡ HS-FFQ, high school food frequency questionnaire; RE, retinol equivalents.

§ Postprandial rise in serum glucose level compared with the rise in serum glucose for 1 g of reference carbohydrate (white bread).

¶ Each unit of dietary glycemic load represents the glycemic equivalent of 1 g of carbohydrate from white bread.

recalled diet (for nutrients, $r = 0.20$) suggest that our reproducibility results were not substantially inflated by current adult diet. The timing of assessment of current diet, whether before or after the administration of the HS-FFQ, also did not influence the results.

Other investigators have reported a larger correlation between current diet and remotely recalled diet (26–29). For instance, Bakkum et al. (27) reported a 0.72 food correlation for men and a 0.64 correlation for elderly men and women. Wu et al. (26) reported food correlations of 0.54 for men and 0.56 for women. One explanation for this difference is that in some studies, participants' current diets were assessed at the same time as their recalled diets, which could have influenced recall and artificially inflated their results due to correlated error (26, 27, 29). Alternatively, these reports may truly reflect stability of adult diet over time.

Our low correlations between current diet and recalled diet, together with the stronger correlations between two recalls of high school diet, suggest that participants may have eaten differently during high school (14). For instance, the greatest decrease in nutrients was for fats; there was a 60 percent decline in total saturated fat intake, which is consistent with national trends. Reported calories from total fat also decreased from 40 percent to 29 percent, and calories from carbohydrates and protein increased. Because of presumed diet stability, some authors have suggested that current diet be used as a surrogate measure of past diet (7, 29). However, our results imply that the best measure of past adolescent diet (in the absence of original data) is recalled, not current, diet—a conclusion consistent with previous investigations (8, 14, 30).

Dietary data may be prone to systematic underreporting of food and nutrient intakes and, to a lesser extent, systematic overreporting (21, 31). We did not find evidence for underreporting using the Goldberg cutoff for the ratio of energy intake to basal metabolic rate. Furthermore, our analysis did not indicate appreciable differences in the correlations for subjects classified as "underreporters," "high reporters," and "acceptable reporters" with the use of 20 percent cutoffs for energy intake.

Correlated error is probably present between the two NHSII HS-FFQ reports, which will tend to produce overestimation of the reproducibility correlations. This underlies the importance of having an independent estimate of intake, which was our intention in comparing mothers' reports with their daughters' reports in this study.

Maternal comparison

The correlations for the maternal comparison were modest for foods and moderate for nutrients. Other studies reported similar or weaker results. Wolk et al. (25) examined adolescent diet recalled by study participants with the adolescent diet remotely recalled by their adult siblings as a proxy external comparison. The average correlation was 0.30 for foods and nutrients. Several other studies have examined the validity of distant diet (>10 years), comparing diet that was recalled with diet recorded at the time of interest (often called original diet); these studies have been reviewed elsewhere (10, 14). Dwyer et al. (32) examined the validity of

adolescent diet using diet histories recorded in childhood and found a low median nutrient correlation of 0.12 for recalled foods eaten at age 18 years. This low correlation could be due to the rather crude original assessment of diet. Other studies addressing the validity of diet during adulthood (recalled 11–24 years in the past) have reported average correlations that were moderate for food intakes (range of average correlations, 0.29–0.40) and higher for nutrient intakes (range of average correlations, 0.23–0.59) (26–29, 33, 34). Although we did not have data on original diet, our correlations appear to be consistent with these reports.

Correlated error between NHSII reports and mothers' reports could have led to overestimation of validity if, for example, the mothers discussed their responses with their daughters before returning the questionnaire. We took precautions to minimize this possibility (as detailed in the Materials and Methods section). Although we cannot completely exclude this bias, we believe it is unlikely that a large portion of mothers ignored our instructions and discussed their responses with their daughters. In addition, the daughters had completed the questionnaires more than 4 years earlier, and it is unlikely that they remembered specific responses.

An important limitation of this study is that we did not have actual diet information obtained from participants when they were in high school. The mothers' reports provided some measure of validity, although not a perfect one (35). The fact that mothers may not have been aware of all their nurse-daughters' food habits outside the home would have resulted in error in reporting. For instance, the mothers tended to underreport caffeine and fat more than fruit- and vegetable-related nutrients in comparison to the NHSII participants' reports, which supports the idea that they did not know all that their daughters were eating. In addition, mothers' fading memories may have also contributed to error in reporting of diet, which would have attenuated correlations. In making this comparison between mothers' and daughters' reports, we recognize that there are virtually no true measures of absolute intake for adolescent diet decades in the past, only imperfect standards. This underscores the methodological challenges of evaluating retrospective recall of diet in the distant past. In the absence of actual diet information from the NHSII participants, a more rigorous validation study would be desirable—for example, administration of the same questionnaire to a group of participants for whom diet was recorded when they were in high school.

Lastly, the NHSII participants represented in the reproducibility and maternal comparison components of this study consisted largely of Caucasian women. Thus, these findings are not necessarily generalizable to men or to other women with different ethnic backgrounds, age, or education. However, our study subsample was representative of the full NHSII cohort with respect to age, body mass index (weight (kg)/height (m)²), smoking status, and reproductive variables.

This study also had several advantages over other investigations of this topic. First, it is one of the few that has examined diet during high school. Adolescence may be a particularly important time for the study of chronic diseases,

and this remains a relatively unexplored area of investigation. The period of time between repeated questionnaire administrations was long enough (4 years) that it is unlikely that participants would have remembered their initial responses and been influenced by them in the second administration. The potential of recall bias in estimating food and nutrient intakes underlies the importance of prospective studies, such as the present study, in which data are collected before disease occurs. Lastly, as an implication of our results, the low correlations between recalled high school diet and current diet suggest that our information on high school diet was almost independent of data on adult diet; thus, the high school dietary information from it has the potential to add new insights on disease etiology. While further studies are warranted, our findings suggest that this food frequency questionnaire, completed in adulthood, provides a reasonable record of diet during adolescence for use in assessing associations with adult disease.

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Adult Recall of Adolescent Diet: A Validation Study

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Diet during earlier periods of life, such as in adolescence, is of considerable research interest because of its potential relevance to the development of chronic disease. Though retrospective recall of adolescent diet can provide a timely way to assess diet-disease relations until current prospective studies reach clinical endpoints, few studies have assessed the validity of adolescent diet as recalled many years later by adults.

In this investigation, we examined the validity of a 124-item food frequency questionnaire (HS-FFQ) used to ask 80 young adults in the United States about their diets during high school. We compared the HS-FFQ with original diet data collected when the participants were actually of high school age - three 24-hour recalls and two repeated food frequency questionnaires (YAQ) collected 10 years earlier in 1993 when the participants were approximately between 13 to 18 years old. We used Pearson correlation coefficients to compare 20-25 nutrients as reported on the 24-hour recalls, YAQ, and HS-FFQ and corrected these Pearson correlations for within-person variation in the replicate measure, using deattenuation correction procedures.

The average corrected correlation for nutrient intakes calculated from the HS-FFQ and the 24-hour recalls was 0.45 (range: 0.16 - 0.69). The corrected correlations in our comparison between the HS-FFQ and YAQs were only modestly higher than these results (mean $r=0.58$, range 0.40 - 0.88). Lastly, the average deattenuated correlation between the 24-hour recalls and the mean of the two YAQs was 0.69 (range: 0.45 - 0.97).

Taken together with our earlier work, this analysis suggests that a food frequency questionnaire used to assess diet retrospectively during high school is sufficiently valid to be of value in the study of diet and disease relationships.