



## ORIGINAL CONTRIBUTIONS

### Caregiving Stress, Endogenous Sex Steroid Hormone Levels, and Breast Cancer Incidence

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Stress is hypothesized to be a risk factor for breast cancer. The authors examined associations of hours of, and self-reported levels of stress from, informal caregiving with prospective breast cancer incidence. Cross-sectional analyses of caregiving and endogenous sex steroid hormones were also conducted. In 1992 or 1996, 69,886 US women from the Nurses' Health Study, aged 46–71 years at baseline, answered questions on informal caregiving; 1,700 incident breast cancer cases accrued over follow-up to 2000. A subset of 665 postmenopausal women not taking exogenous hormones returned a blood sample in 1990. Numbers of hours of care provided to an ill adult or to a child were each summed and analyzed as 0 (reference), 1–14, and  $\geq 15$  per week. Cox proportional hazards models were used in prospective analyses and linear models in cross-sectional analyses. High numbers of caregiving hours and self-reported stress did not predict a higher incidence of breast cancer. However, compared with women providing no adult care, women providing  $\geq 15$  hours of adult care (median, 54) had significantly lower levels of estradiol (geometric mean, 9.21 pg/ml vs. 7.46 pg/ml (95% confidence interval: 6.36, 8.76)) and bioavailable estradiol (geometric mean, 1.86 pg/ml vs. 1.35 pg/ml (95% confidence interval: 1.00, 1.82)). Stress from caregiving did not appear to increase breast cancer risk.

breast neoplasms; caregivers; cohort studies; gonadal steroid hormones; stress, psychological

Psychosocial stress has long been implicated in the development of breast cancer, although the empirical findings have been mixed (1–8). Most studies of stress and cancer risk have measured stress in terms of specific adverse life events (bereavement, job loss, divorce, disasters, or family illness or death) (9), but a meta-analysis by Petticrew et al. (10) concluded that recent adverse life events are not causative factors in the development of breast cancer. However, adverse life events differ from daily chronic stressors that may persist indefinitely (11). One study examining both acute and chronic stress found that chronic stress, but not acute stress, was related to breast cancer relapse (12).

Caregiving has been reported to be stressful (13–15). Stress associated with caregiving can persist indefinitely and may have pronounced effects on health (16–18). No prior study, to the authors' knowledge, has examined stress from

informal caregiving activities and cancer risk. Therefore, we hypothesized that chronic stress from prolonged, full-time, informal caregiving would be prospectively associated with an increased risk of breast cancer. Because subjective response to stress may be as or more relevant than the objective stressor to outcomes (19), we further hypothesized that high self-reported stress from caregiving would predict a higher risk of breast cancer. We also hypothesized that other potential psychosocial stressors such as full-time work, social isolation, and depressive symptoms would exacerbate the effects of caregiving stress on the rate of cancer incidence.

Endogenous reproductive hormones have been strongly implicated in the etiology of breast cancer; prospective studies of postmenopausal women show an increased risk with elevated estrogen levels (20–22). Consistent with the

above hypotheses, we hypothesized that women providing high levels of caregiving would have higher levels of estrogens.

## MATERIALS AND METHODS

### Nurses' Health Study subjects and blood sample collection

The Nurses' Health Study is a longitudinal study of 121,700 US female nurses, 30–55 years of age at baseline in 1976. At baseline and during biennial follow-up periods, participants provided detailed lifestyle and medical history information through a mailed questionnaire.

We included in this study women who answered questions on informal caregiving activities in 1992 or 1996. Women with prior cancer ( $n = 9,274$ ) and cardiovascular disease (myocardial infarction, angina, stroke) ( $n = 6,080$ ) were excluded from the study.

To ensure the proper time order between exposure and outcome, we attempted to exclude fatal breast cancer cases between 1992 and 1996; however, after other exclusions, no women with incident breast cancer died from the disease within this 4-year period. After exclusions, 55,395 women, aged 46–71 years in 1992, were included in the analysis (table 1). Of these women, 759 were diagnosed with breast cancer between 1992 and 1996.

For analyses with follow-up from 1996 to 2000, we also excluded 2,590 women who had cancer between 1992 and 1996, 2,172 women who had cardiovascular disease, 811 women who died in the interim, and two women who were diagnosed with breast cancer after 1996 and died within the 4-year follow-up period. A remaining 55,430 women were included in analyses, and 941 were diagnosed with invasive breast cancer between returning the 1996 questionnaire and June 2000. In total, 69,886 women were included in the study, and 1,700 new cases of primary invasive breast cancer accrued between 1992 and 2000.

Women included in the analysis of endogenous hormones ( $n = 665$ ) were controls in a nested case-control study of plasma hormone levels and breast cancer risk and responded to questions on informal caregiving in 1992 (23). Women included were postmenopausal (no menses for at least 12 months before blood sampling) and had not used hormones for at least 3 months before the blood collection. Participants had no previously diagnosed cancer (except nonmelanoma skin cancer).

### Data collection

Blood samples were collected in 1989–1990 from 32,826 Nurses' Health Study participants 43–69 years of age at the time, as detailed previously (24–26). Hormone levels for the plasma samples used in this analysis were assayed in four batches between 1990 and 1998.

We further measured hours of informal caregiving (unrelated to participants' paid work as nurses) and self-reported caregiving stress. In 1992 and 1996, Nurses' Health Study participants were asked how many hours per week they provided care for each of specific categories of kin including

children, grandchildren, a disabled or ill spouse, a disabled or ill parent, or another disabled or ill person. Participants could respond that they provided care for each person 0 (reference category), 1–8, 9–20, 21–35, 36–72, or 73 or more hours per week.

Participants were further asked, "How stressful would you say it is to provide care to the individuals mentioned above?" Choices included not applicable, not at all, just a little bit, moderately, extremely, and don't know.

In 1992 and 1996, depressive symptoms were assessed by using the five-item Mental Health Index (MHI-5) from the Medical Outcomes Study Short-Form 36 (SF-36) Health Status Survey. A score of 52 or less (on a scale of 0–100) on the short-form mental health scale was used to define the group with depressive symptoms (27). Social networks were measured by using the Berkman-Syme Social Networks Index (28). Responses to this index were categorized into four levels of social connection from low to high (29). Participants were also asked about their working status, indicating whether they were employed full time or part time (as a nurse or in another profession), worked as a homemaker, or were retired.

Data on other biomedical, lifestyle, and hormonal factors have also been assessed, including age, alcohol consumption, body mass index, physical activity, husband's education, recent mammography, family history of breast cancer, benign breast disease, past oral contraceptive use, age at menarche, age at first birth, parity, age at menopause, type of menopause, and postmenopausal hormone use. We controlled for these variables in multivariate-adjusted analyses.

In the Nurses' Health Study, incident breast cancer was ascertained by a biennial mailing of the questionnaire to participants. For any report of cancer (except basal cell skin cancer), written permission was obtained from study participants to review their medical records. Physicians, blinded to exposure information from questionnaires, subsequently reviewed medical records and pathology reports. Overall, 99 percent of self-reported breast cancers for which medical records are obtained have been confirmed.

Ascertainment of deaths in the Nurses' Health Study cohort has included reporting by the family or postal authorities. Additionally, names of persistent nonresponders are searched in the National Death Index. More than 98 percent of deaths in the Nurses' Health Study cohort have been identified by this method (30).

### Laboratory analysis

Hormone fractions of estradiol, estrone, estrone sulfate, and testosterone were assayed in four different batches. Estrone sulfate from batches 1 and 2 was assayed in the laboratory of Dr. C. Longcope (University of Massachusetts Medical Center, Worcester, Massachusetts). All other analyses were performed by the Nichols Institute (San Juan Capistrano, California). Methods for plasma hormone assays and detailed information regarding laboratory precision and reproducibility have been published previously (23, 31, 32). Within-batch laboratory coefficients of variation were all 15 percent or less.

**TABLE 1. Selected characteristics, by categories of caregiving hours in 1992, of 55,395 women\* from the US Nurses' Health Study**

	No. of hours per week of informal caregiving in 1992							
	Caregiving to an adult				Caregiving to a child			
	0 (reference)	1–14	≥15	<i>p</i> for linear trend	0 (reference)	1–14	≥15	<i>p</i> for linear trend
No. of subjects	43,424	9,566	2,405		32,059	17,586	5,750	
Mean age (years)†	58.3	57.8	59.2	<0.01	59.4	57.3	54.8	<0.01
Median no. of hours of care provided to an adult	0	4.5	54.0		0	0	0	
Mean no. of hours of care provided to children	0	4.5	4.5		0	4.5	45.2	
Reproductive factors								
Age at menarche (% <13 years)	48.4	48.4	51.5	<0.01	48.8	48.4	47.2	0.02
Parous (%)	92.5	91.8	90.8	<0.01	90.1	95.4	94.9	<0.01
Ever oral contraceptive use (%)	53.2	51.6	50.1	<0.01	53.9	52.4	47.9	<0.01
Age (years) at first birth‡	24.8	24.7	24.6	0.09	24.6	24.7	25.4	<0.01
No. of children‡	3.1	3.1	3.2	0.02	2.9	3.3	3.6	<0.01
Postmenopausal (%)	83.1	83.7	86.0	<0.01	84.4	83.1	78.1	<0.01
Age (years) at menopause§	46.4	46.6	46.4	0.99	46.4	46.6	46.5	0.59
Natural menopause (%)§	57.2	57.7	57.1	0.90	57.0	57.8	57.3	0.75
Postmenopausal hormone use (%)§	42.8	41.8	39.8	<0.01	44.1	41.7	33.7	<0.01
Birth index¶	44.7	45.0	43.5	0.13	41.4	49.2	49.1	<0.01
Breast cancer factors								
Family history (%)	11.6	11.9	10.8	0.25	11.8	11.2	11.6	0.74
Benign breast disease (%)	44.3	45.1	44.0	0.77	45.8	44.1	37.9	<0.01
Mammogram screening (%)	83.3	83.3	79.4	<0.01	84.2	82.9	77.7	<0.01
Lifestyle factors								
Full-time work (%)	38.8	36.5	33.4	<0.01	40.2	38.4	26.2	<0.01
Alcohol consumption (g/day)	5.0	4.5	3.9	<0.01	5.3	4.4	3.6	<0.01
Current smoking (%)	13.9	13.5	16.3	<0.01	14.2	13.3	14.4	0.64
Body mass index (kg/m <sup>2</sup> )	25.9	26.1	26.6	<0.01	25.7	26.2	26.6	<0.01
Physical activity (METs#/week)	19.4	19.9	20.4	0.03	19.9	18.9	19.1	0.01
Psychosocial factors								
Depressive symptoms (%)	6.8	8.3	15.8	<0.01	6.9	7.9	9.1	<0.01
Low social network (%)	5.7	3.2	4.9	0.09	6.2	4.1	3.3	<0.01
Husband ≥college education (%)	46.6	46.2	41.4	<0.01	46.7	45.7	45.3	0.05
High stress, self-report (%)	13.3	38.7	73.2	<0.01	17.1	20.6	36.6	<0.01

\* Total subjects in study: *n* = 69,886 (1,700 breast cancer cases); subjects for 1992 analyses: *n* = 55,395 (759 cases); subjects for 1996 analyses: *n* = 55,430 (941 cases).

† Except for age, all variables were adjusted for age.

‡ Among 51,121 parous women.

§ Among 46,155 postmenopausal women.

¶ Adjustment for the birth index enables adjustment for parity and age at each birth with a single, continuous variable.

# METs, metabolic equivalents.

### Statistical analyses

To obtain continuous caregiving hours for each kin group, the numerical values 0, 4.5, 14.5, 28, 54, and 73 were assigned to the respective categorical responses mentioned above (0, 1–8, 9–20, 21–25, 36–72, and ≥73 hours per

week). Total hours of care provided to an ill adult were then obtained by summing numbers of hours of informal care given to an ill spouse, parent, or other adult. Total hours of care provided to a child were correspondingly obtained by summing numbers of hours of informal care given to a child or grandchild. For categorical analyses of these new vari-

ables, continuous variables were collapsed to 0, 1–14, and 15 or more hours. We chose these categories because of the low frequency of women indicating more than 15 hours of caregiving per week.

When exploring combined effects of stress and other variables, hours were categorized as 0 and as more than 0 per week in stratified analyses. Social networks were divided into high networks (top two categories) and low networks (bottom two categories). We also computed the change in social networks between 1992 and 1996 and created three categories: declining networks, stable networks, and improving networks. Work status was collapsed to full-time work and other.

Different types of caregiving may have differential effects on health (33) and on stress levels. Therefore, we analyzed adult and child caregiving separately, adjusting simultaneously for numbers of adult and child caregiving hours. Furthermore, hours of care provided to different kinship groups (children, grandchildren, parent, spouse, other) were analyzed simultaneously in models.

We used Cox proportional hazards models (the SAS PROC PHREG procedure) (34) for failure-time data to simultaneously calculate point and interval estimates of hazard rate ratios for more than one exposure variable of interest and to evaluate and quantify interaction terms (35, 36). Person-months of follow-up were counted from the date a psychosocial questionnaire was returned (in 1992 or 1996) until the date of death, breast cancer event, or age at end of follow-up, whichever came first. Relative risk estimates were obtained by exponentiation of the fitted  $\beta$  to a particular model; 95 percent confidence intervals were obtained by exponentiation of the 95 percent confidence bounds of  $\beta$ . Departures from the proportional hazards assumption were tested by using likelihood ratio tests comparing models with and without the time-by-covariate interaction terms.

Because the latency period of a stress exposure is unknown, we first conducted analyses by assigning the most recent exposure status to subsequent outcomes (1992 caregiving with outcomes between 1992 and 1996, and 1996 caregiving with outcomes between 1996 and 2000). We subsequently conducted a lagged analysis of 1992 caregiving with breast cancer between 1996 and 2000. To evaluate whether chronic stress from caregiving was the relevant exposure, 1996–2000 cancer outcomes were regressed on cumulative exposure assessed as the average number of hours of adult or child caregiving provided in 1992 and 1996. This approach has the further advantage of reducing random error in the exposure (37).

We further regressed outcomes on self-reported stress from child or adult caregiving responsibilities represented in terms of indicator variables, collapsed to the following categories: not providing any care (reference); not at all or just a little bit stressful; moderately or extremely stressful; and missing, not applicable, or don't know. We also explored stratified associations by levels of self-reported stress—high and moderate versus little or none.

In additional models, we stratified by levels of social networks, depressive symptoms, and employment status to examine possible effect modification. Interaction terms were computed for linear hours and the dichotomous versions of

each of these variables. Because social support may be an important buffer of the stress response (38, 39), we also compared whether change in social support (increasing, decreasing, or staying the same), adjusted for baseline social support, buffered the association between caregiving hours and breast cancer outcome.

Age and age at menopause were analyzed continuously in models. Age-adjusted results were compared with those obtained by adjusting for multiple reproductive, lifestyle, and psychosocial factors (table 2). We also adjusted for birth index, a variable developed by Colditz and Rosner (40). The birth index =

$$\sum_{i=1}^s (t^* - t_i) b_{it},$$

where  $t^*$  = minimum(age, age at menopause);  $t_i$  = age at the  $i$ th birth;  $i = 1, \dots, s$ ; and  $b_{it} = 1$  if parity  $\geq i$  at age  $t$  or 0 otherwise. Adjustment for this variable enables fine adjustment for parity and age at each birth with the inclusion of a single, continuous variable.

To include women for whom information was missing and to reduce bias, we used the Greenland and Finkle (41) regression method in analyses of certain continuous caregiving variables and for continuously analyzed reproductive covariates. Missing values were assigned an arbitrary value, and a missing-value indicator was created; it took the value 1 wherever the original variable was missing and 0 otherwise. An advantage of this method is an improved ability to adjust for potential confounding. For categorical covariates, we also created indicator variables to represent missing covariate information.

For hormonal biomarker values that were less than the limit of sensitivity, we reset values to minimum plausible levels (estrone = 5, estrone sulfate = 20, testosterone = 1, progesterone = 1.5). Then, using linear regression (the SAS PROC GLM procedure; SAS Institute, Inc.), we regressed log mean levels of reproductive hormones including estradiol, bioavailable estradiol, estrone, and progesterone on categories of both adult and child caregiving hours. We included variable numbers of women in analyses since the number of those for whom data on specific hormones were available varied. These analyses were adjusted for age, body mass index, laboratory, time since blood draw, smoking, husband's education, and age at menarche. All tests of statistical significance were two-sided.

## RESULTS

Age-adjusted distributions of multiple covariates are presented, by categories of adult and child caregiving hours, in table 1. In 1992, 11,971 (22 percent) women reported providing informal adult care, and 23,336 (42 percent) supplied informal child care. A total of 2,405 (4 percent) women provided 15 or more hours of adult care, and 5,750 (10 percent) provided 15 or more hours of care to a child. Those supplying 1–14 hours of care to a child or adult provided a median of 4.5 hours of care per week. Those providing 15 or more hours a week of adult care reported a

**TABLE 2. Relative risk (95% confidence interval) of incident breast cancer, by categories of caregiving hours, for 69,886 women\* from the US Nurses' Health Study**

	No. of hours per week of informal caregiving							
	Caregiving to an adult				Caregiving to a child			
	0 (reference)	1–14	≥15	<i>P</i> value†	0 (reference)	1–14	≥15	<i>P</i> value†
No., 1992	43,424	9,566	2,405		32,059	17,586	5,750	
No. of cases, 1992–1996	607	125	27		451	241	67	
No., 1996	43,627	9,546	2,257		35,555	16,060	3,815	
No. of cases, 1996–2000	739	161	41		641	237	63	
Recent caregiving								
Age adjusted	1.00	1.00 (0.88, 1.14)	0.93 (0.73, 1.19)	0.40	1.00	0.92 (0.83, 1.03)	0.96 (0.80, 1.15)	0.89
Multivariate adjusted‡	1.00	0.99 (0.87, 1.13)	0.94 (0.73, 1.20)	0.44	1.00	0.94 (0.84, 1.05)	1.01 (0.84, 1.22)	0.68
Lagged analysis, 1992 caregiving§								
Age adjusted	1.00	0.95 (0.77, 1.17)	1.06 (0.74, 1.53)	0.60	1.00	0.84 (0.71, 1.00)	0.96 (0.73, 1.25)	0.29
Multivariate adjusted‡	1.00	0.95 (0.78, 1.17)	1.06 (0.74, 1.53)	0.59	1.00	0.86 (0.73, 1.02)	1.01 (0.77, 1.32)	0.48
Cumulative average, 1992 and 1996 hours§								
Age adjusted	1.00	1.07 (0.90, 1.27)	1.19 (0.87, 1.62)	0.22	1.00	0.83 (0.71, 0.97)	0.83 (0.63, 1.10)	0.46
Multivariate adjusted‡	1.00	1.07 (0.90, 1.27)	1.19 (0.87, 1.62)	0.21	1.00	0.85 (0.72, 1.00)	0.87 (0.66, 1.16)	0.69

\* Total subjects in study:  $n = 69,886$  (1,700 breast cancer cases); subjects for 1992 analyses:  $n = 55,395$  (759 cases); subjects for 1996 analyses:  $n = 55,430$  (941 cases).

† For continuous variable.

‡ Multivariate-adjusted models were adjusted for age (continuous), alcohol consumption (g/day; 0 (reference), >0–14.9, 15.0–24.9, ≥25.0), body mass index (kg/m<sup>2</sup>; <21, 21–22 (reference), 23–24, 25–29, ≥30), physical activity (metabolic equivalents (METs)/week; <3 (reference), 3–17, ≥18), work status (not employed (reference), nursing position, nonnursing position), husband's education (college graduate (no (reference), yes)), social networks (most integrated (reference), moderately integrated, moderately isolated, most isolated), depressive symptoms (no (reference), yes), recent mammography (no (reference), yes), family history of breast cancer (no (reference), yes), benign breast disease (no (reference), yes), age at menarche (years; 7–11, 12 (reference), 13, 14, 15–21), past oral contraceptive use (no (reference), yes), birth index (continuous), age at menopause (continuous), type of menopause (premenopausal, natural menopause (reference), surgery but no ovaries removed, one ovary removed, two ovaries removed), and postmenopausal hormone use (never (reference), past, current).

§ For lagged analysis, 1996–2000 cases were regressed on 1992 caregiving; for analysis of the cumulative average number of caregiving hours, 1996–2000 cases were regressed on the average number of 1992 and 1996 caregiving hours.

median of 54 hours of care per week. Women providing adult care also tended to administer some child care (table 1).

Women providing 15 or more hours of care per week to an adult were older on average and therefore more likely to be postmenopausal. They were also less likely to have ever used oral contraceptives, although parity was no higher than for those providing less or no care. Because of their increased caregiving responsibilities, these women were less likely to be employed full-time outside of the home. They were also more likely to report high levels of stress and depressive symptoms and were more likely to smoke; however, their level of physical activity was slightly higher, they drank less per week, and they were less likely to be socially isolated. Family history of breast cancer was unrelated to caregiving. In contrast, compared with women providing no child care, those providing 15 or more hours of caregiving to a child were younger and had more children (table 1).

One fourth of the women in the cohort provided care to their own children in 1992, and another 25 percent indicated doing so for grandchildren. In 1996, about half as many (14 percent) provided care to their children, but slightly more (28 percent) provided care to grandchildren. Fewer supplied care to an ill adult, although the proportion providing care to an ill

spouse (~5 percent), parent (~12 percent), or other adult (~9 percent) was approximately the same in both time periods (data not shown).

For those providing care, numbers of hours of care given to children, grandchildren, parents, or "other" ill adults remained approximately the same between 1992 and 1996 (median, 4.5 hours/week for each group at each time point). In contrast, spousal caregiving responsibilities increased from a median of 4.5 hours in 1992 to 14.5 hours in 1996. Among those at or above the 75th percentile of caregiving hours, the caregiving burden nearly doubled from 28.0 to 54.0 hours (data not shown).

In both age-adjusted and multivariate-adjusted analyses of caregiving, hours of care provided to an ill adult and to a child were not associated with breast cancer incidence (table 2). Results were generally similar whether the caregiving exposure was considered recent, lagged, or cumulative. The exception was that a lower incidence of breast cancer was found between 1996 and 2000 for those providing on average 1–14 hours of care to a child (0 hours: reference; 1–14 hours: hazard ratio = 0.85, 95 percent confidence interval: 0.72, 1.00; and ≥15 hours: hazard ratio = 0.87, 95 percent confidence interval: 0.66, 1.16), although the trend was

**TABLE 3. Relative risk (95% confidence interval) of incident breast cancer, by categories of self-reported stress, for 69,886 women\* from the US Nurses' Health Study**

	Self-reported stress level†		
	Not providing any caregiving (reference)	Not at all or a little bit stressful	Moderately or extremely stressful
No., 1992	43,424	6,150	5,475
No. of cases, 1992–1996	607	89	61
No., 1996	43,627	6,415	5,057
No. of cases, 1996–2000	739	118	79
Adult caregiving‡	1.00	1.11 (0.95, 1.29)	0.82 (0.68, 1.00)
No., 1992	451	197	79
No. of cases, 1992–1996	32,059	15,392	5,829
No., 1996	641	214	63
No. of cases, 1996–2000	35,555	14,389	3,950
Child caregiving‡	1.00	0.90 (0.80, 1.01)	1.12 (0.92, 1.36)

\* Total subjects in study:  $n = 69,886$  (1,700 breast cancer cases); subjects for 1992 analyses:  $n = 55,395$  (759 cases); subjects for 1996 analyses:  $n = 55,430$  (941 cases).

† Numbers of subjects in a fourth category that included don't know, not applicable, or missing varied from 331 to 2,115.

‡ Models were adjusted for all factors described in table 2 (multivariate-adjusted models).

nonsignificant overall ( $p$  value, continuous variable = 0.69). Caregiving to specific kinship groups (child, grandchild, parent, spouse, or other ill adult) was also unrelated to the outcome (data not shown). Furthermore, no significant interactions were found between caregiving and other psychosocial variables.

High levels of self-reported stress from caring for a child were unrelated to breast cancer outcome. However, high levels of self-reported stress associated with adult care were related to a borderline lower incidence of breast cancer (hazard ratio = 0.82, 95 percent confidence interval: 0.68, 1.00) (table 3).

Compared with women providing no adult care, women providing 15 hours or more per week of care had significantly lower mean levels of estradiol (9.21 pg/ml vs. 7.46 pg/ml, 95 percent confidence interval: 6.36, 8.76;  $p$  for trend < 0.01) and bioavailable estradiol (1.86 pg/ml vs. 1.35 pg/ml, 95 percent confidence interval: 1.00, 1.82;  $p$  for trend = 0.03) (table 4). They also had lower levels of testosterone. On the other hand, caregiving provided to children was positively associated with prolactin ( $p$  for trend < 0.01). Numbers of hours of caregiving provided to an adult or child were unrelated to levels of estrone or progesterone. Self-reported stress and depressive symptoms were unrelated to levels of any of the endogenous hormones (data not shown).

## DISCUSSION

Contrary to our initial hypothesis, high numbers of caregiving hours and high self-reported stress did not predict a higher incidence of breast cancer. In cumulative analyses, moderate time spent providing caregiving to children and high self-reported stress from adult caregiving responsibilities were inversely related to prospective breast cancer incidence. Furthermore, high numbers of hours of adult caregiving were related to lower levels of certain endogenous sex steroid hormones, portending a possible lower future risk of breast cancer.

Stress is thought to influence development of cancer through immune down-regulation. Acting via the hypothalamic-pituitary-adrenal axis in a complex feedback loop between the central nervous and immune systems, stress increases cortisol levels (42–44), which adversely affects immune function (14, 45–56). Frequent cortisol release that occurs with chronic stress may lead to persistently high cortisol levels (57), thus leading to immune suppression. Caregiving has been linked to lower levels of interleukin-2, natural killer cell activity, and other immune parameters (58–61). It has been hypothesized that because the immune system is invoked in eliminating mutated cells, reduced immunity could lead to more rapid development of cancer. Stress might also promote cancer through DNA damage, faulty DNA repair, inhibition of apoptosis, effects on endocrine parameters, or somatic mutation (62). These factors

**TABLE 4. Geometric mean values of several reproductive hormones, by category of caregiving hours ( $n = 422-645$ ), for women from the US Nurses' Health Study\***

	No. of hours per week of informal caregiving							
	Caregiving to an adult†				Caregiving to a child†			
	0 (reference)	1-14	≥15	$p$ for linear trend	0 (reference)	1-14	≥15	$p$ for linear trend
No. of subjects	485	127	36		319	141	188	
Estradiol (pg/ml)	9.21	9.58	7.46	<0.01	8.08	8.85	9.21	0.06
Bioavailable estradiol (pg/ml)	1.86	2.12	1.35	0.03	1.52	1.88	1.84	0.10
Prolactin (ng/ml)	9.21	9.12	9.03	0.78	8.41	8.67	10.38	<0.01
Testosterone (ng/dl)	20.09	21.12	15.49	<0.01	18.92	18.54	18.73	0.99

\* Blood samples were collected in 1989-1990, and study participants provided information about numbers of hours of informal caregiving in 1992.

† Models were adjusted for age (years; <50, 50-54, 55-59, 60-64 (reference), ≥65), body mass index (quintiles (quintile 2 = reference)), laboratory, time of blood draw (months prior; 0, 1-8, 9-12 (reference), 13-16, 17-24), current smoking (no (reference), yes), age at menarche (years; <12 years (reference), 12, 13, ≥14), and husband's education (college graduate (no (reference), yes).

may be precursors to certain types of cancer such as hormonal (63, 64) and lymphatic (7, 9) cancers. However, despite the plausibility of these mechanisms, little epidemiologic evidence (65), including results from this study, exists to support the hypothesis that stress in a healthy population is related to breast cancer.

It is possible that life stress is of insufficient magnitude to affect cancer unless the immune system is already compromised (46), which may explain findings of stress and lowered survival after breast cancer diagnosis (12). This hypothesis is consistent with findings by Vitaliano et al. (66). These authors concluded that psychosocial stress was not associated with levels of natural killer cell activity in spousal caregivers of Alzheimer's disease patients but was associated in the presence of a history of cancer. In our study, little evidence existed that a combination of stressors was any more predictive. Of course, the stressors we examined may be insufficient to lead to the kind of immune down-regulation found in those diagnosed with cancer.

Null associations of psychosocial stress with breast cancer outcomes could reflect competing effects of both reduced immunity and reduced exposure to estrogen. Recent studies have found that low socioeconomic status and depression suppress estrogen levels and lead to early menopause (67-69), factors related to a lower risk of breast cancer. To the extent that immune down-regulation adversely affects breast cancer outcomes, a corresponding decline in estrogens could obscure apparent effects through this mechanistic pathway. Little research has examined the potential effects of stress per se on endogenous hormones, but our results are consistent with prior literature. The magnitude of the difference between women with and without a history of depression in Harlow et al.'s study (67) was similar to that in our study

comparing women providing no adult caregiving with those providing high levels of care.

Despite the logic that reduced immune function may predict higher levels of breast cancer, the effects of immunosuppression on breast cancer etiology may be counterintuitive. Although not definitive, the findings from one study (70) showed that immunosuppression in women after organ transplant was associated with a lowered risk of breast cancer over a 1- to 11-year follow-up (relative risk = 0.75,  $p = 0.009$ ); in another study (71), women with acquired immunodeficiency syndrome-related immunosuppression also had a lower risk of breast cancer (relative risk = 0.5, 95 percent confidence interval: 0.3, 0.8).

Rather than predicting an elevated risk of breast cancer, these findings and mechanistic data from previous studies suggest that high levels of stress could ultimately be associated with a lower rather than a higher risk of breast cancer. Our largely null findings for caregiving and breast cancer may therefore be due to the relatively short-term follow-up (8 years since the first psychosocial assessment and only 4 years since the 1996 assessment). From time of initiation, cancers are thought to progress to a diagnosable malignant tumor over 15-20 years. For breast cancer, the estimated median growth time to detection is 7-11 years (72, 73). We would expect to detect an association between stress and incident breast cancer if the effects of stress served to promote (or inhibit) a tumor from an earlier stage to diagnosable size within 4-8 years.

In this study, high stress from caregiving was not associated with increased prospective cumulative incidence of breast cancer. This finding does not preclude the possible role of stress earlier in life in breast cancer etiology.

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