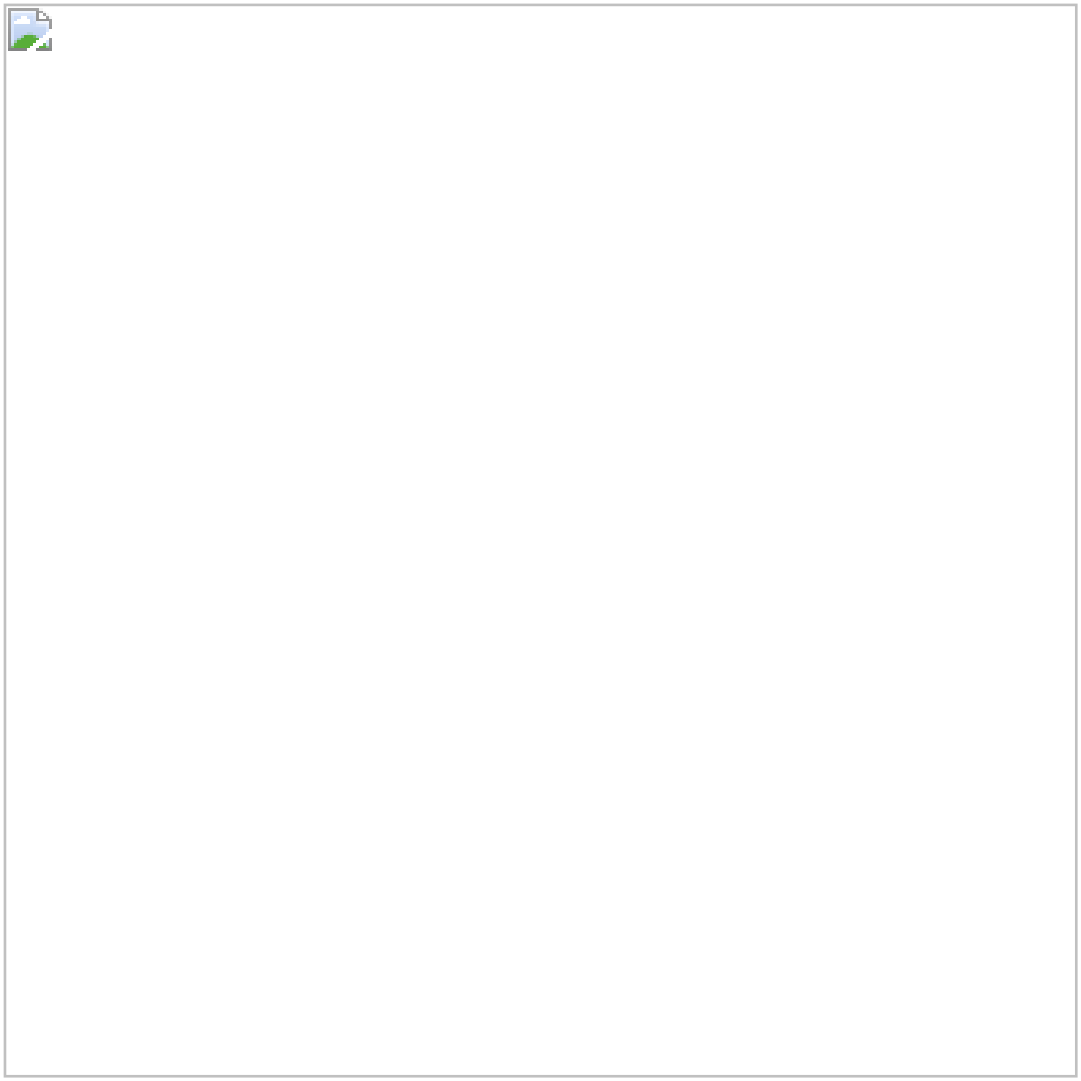

Associations of Ionizing Radiation and Breast Cancer-Related Serum Hormone and Growth Factor Levels in Cancer-Free Female A-Bomb Survivors

Authors: Grant, Eric J., Neriishi, Kazuo, Cologne, John, Eguchi, Hidetaka, Hayashi, Tomonori, et al.

Source: Radiation Research, 176(5) : 678-687

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR2631.1

RADIATION RESEARCH **176**, 678–687 (2011)
0033-7587/11 $15.00

DOI: 10.1667/RR2631.1

Associations of Ionizing Radiation and Breast Cancer-Related Serum Hormone and Growth Factor Levels in Cancer-Free Female A-Bomb Survivors

Eric J. Grant,[a,1] Kazuo Neriishi,[b] John Cologne,[c] Hidetaka Eguchi,[d] Tomonori Hayashi,[e] Susan Geyer,[f] Shizue Izumi,[g] Nobuo Nishi,[h] Charles Land,[i] Richard G. Stevens,[j] Gerald B. Sharp[k] and Kei Nakachi[e]

[a] Department of Epidemiology, [b] Department of Clinical Studies, [c] Department of Statistics, and [e] Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minamiku, Hiroshima 732-0815, Japan; [d] Translational Research Center, International Medical Center, Saitama Medical University, 1397-1 Yamane, Hidaka-shi, Saitama 350-1241, Japan; [f] Department of Internal Medicine, The Ohio State University, Columbus, Ohio 43210; [g] Department of Computer Science and Intelligent Systems, Oita University, 700 Dannoharu, Oita, 870-1192 Japan; [h] Center for Collaboration and Partnership, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan; [i] Radiation Epidemiology Branch, National Cancer Institute, Bethesda, Maryland 20892-7335; [j] Department of Community Medicine, University of Connecticut Health Center, Connecticut 06030; and [k] Epidemiology Branch, Basic Sciences Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Grant, E. J., Neriishi, K., Cologne, J., Eguchi, H., Hayashi, T., Geyer, S., Izumi, S., Nishi, N., Land, C., Stevens, R. G., Sharp, G. B. and Nakachi, K. Associations of Ionizing Radiation and Breast Cancer-Related Serum Hormone and Growth Factor Levels in Cancer-Free Female A-Bomb Survivors. *Radiat. Res.* 176, 678–687 (2011).

Levels of exposure to ionizing radiation are increasing for women worldwide due to the widespread use of CT and other radiologic diagnostic modalities. Exposure to ionizing radiation as well as increased levels of estradiol and other sex hormones are acknowledged breast cancer risk factors, but the effects of whole-body radiation on serum hormone levels in cancer-free women are unknown. This study examined whether ionizing radiation exposure is associated with levels of serum hormones and other markers that may mediate radiation-associated breast cancer risk. Serum samples were measured from cancer-free women who attended biennial health examinations with a wide range of past radiation exposure levels ($N = 412$, ages 26–79). The women were selected as controls for separate case-control studies from a cohort of A-bomb survivors. Outcome measures included serum levels of total estradiol, bioavailable estradiol, testosterone, progesterone, prolactin, insulin-like growth factor-1 (IGF1), insulin-like growth factor-binding protein 3 (IGFBP-3), and ferritin. Relationships were assessed using repeated-measures regression models fitted with generalized estimating equations. Geometric mean serum levels of total estradiol and bioavailable estradiol increased with 1 Gy of radiation dose among samples collected from postmenopausal women (17%$_{1Gy}$, 95% CI: 1%–36% and 21%$_{1Gy}$, 95% CI: 4%–40%, respectively), while they decreased in samples collected from premenopausal women (−11%$_{1Gy}$, 95% CI: −20%–1% and −12%$_{1Gy}$, 95% CI: −20%– −2%, respectively). Interactions by menopausal status were significant ($P = 0.003$ and $P < 0.001$, respectively). Testosterone levels increased with radiation dose in postmenopausal samples (30.0%$_{1Gy}$, 95% CI: 13%–49%) while they marginally decreased in premenopausal samples (−10%$_{1Gy}$, 95% CI: −19%–0%) and the interaction by menopausal status was significant ($P < 0.001$). Serum levels of IGF1 increased linearly with radiation dose (11%$_{1Gy}$, 95% CI: 2%–18%) and there was a significant interaction by menopausal status ($P = 0.014$). Radiation-associated changes in serum levels of estradiol, bioavailable estradiol, testosterone and IGF1 were modified by menopausal status at the time of collection. No associations with radiation were observed in serum levels of progesterone, prolactin, IGFBP-3 or ferritin. © 2011 by Radiation Research Society

INTRODUCTION

Exposure to ionizing radiation via diagnostic imaging, particularly CT scans, continues to increase worldwide (*1*). Numerous studies have proven the association between exposure to ionizing radiation and breast cancer (*2*). Moreover, data from the A-bomb survivors indicate that the breast is particularly susceptible to ionizing radiation exposure, with an attributable fraction for breast cancer of 27% for all women exposed to more than 5 mGy (*3*) [a radiation dose roughly twice the yearly background dose (*4*)]. Breast cancer has also been associated with levels of endogenous hormones, particularly estradiol (E_2) (*5–7*). Estrogens stimulate cell proliferation (*8*) and possibly exert a direct genotoxic effect on breast epithelial cells (*9*).

Other circulating hormones and proteins, including non-sex hormone-binding globulin (SHBG)-bound

Note. The online version of this article (DOI: 10.1667/RR2631.1) contains supplementary information that is available to all authorized users.

[1] Address for correspondence: Department of Epidemiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima 732-0815, Japan; e-mail: egrant@rerf.or.jp.

estradiol, testosterone (*7*) and insulin-like growth factor 1 (IGF1), have been associated with breast cancer (*10*). Additional serum markers have been hypothesized to be associated with breast cancer, including IGFBP-3 (*11*), progesterone (*12*), prolactin (*13*) and ferritin. Ferritin levels and ionizing radiation are associated with increased levels of free radicals, and high ferritin levels have been reported to result in cell transformation and immortalization in mammary glands (*14*).

The question of whether ionizing radiation exposure modifies the underlying levels of risk-related circulating markers has not been addressed, however, and could be important in mechanistic studies of breast cancer or other hormone-related cancers after ionizing radiation exposure. The primary purpose of this project is to examine whether serum levels of risk-related markers vary with past radiation exposure in a representative sample of cancer-free female A-bomb survivors.

MATERIALS AND METHODS

Subjects

Study subjects were selected from the Adult Health Study (AHS), a longitudinal cohort study of A-bomb survivors initiated in 1958 (*15*). The AHS features biennial clinical examinations with serum collection and well-characterized radiation doses for numerous body organs (*16*). Serum samples were selected in the context of concurrent case-control studies wherein controls were selected by incidence density sampling from all subjects who met eligibility requirements and were at risk for a first primary cancer of any type at the time an index case was diagnosed. Eligibility was defined as women who were free from various medical conditions (oophorectomy, current exogenous hormone treatment, prior malignancies, pregnant), were cancer-free for at least 2 years after serum collection, and had estimated radiation organ doses. Hormone-related diseases/conditions were based on self-reports or, in the case of extreme measured values, a medical chart review. Samples were matched to an index case on age at collection (±2 years), sample collection year (same decade), city (Hiroshima or Nagasaki), and counter-matched using breast radiation dose strata (low, medium and high with cut-points at 5 mGy and 790 mGy) (*17*). Counter-matching assures a wide exposure range while still providing an appropriate sample for inference of the exposure effects within the underlying cancer-free cohort (*18*). Because it is not known which organ system might be responsible for changes to serum markers, we used doses to the colon for purposes of analyses. The colon is a centrally located organ, and colon doses have been used as representative doses in other studies (e.g. for overall solid cancer risk estimates), although the results should be relatively insensitive to the organ chosen since the doses to all organs are highly correlated. Radiation doses to the colon were adjusted to account for random errors in the individual radiation dose estimates (*19*). Menopausal status at the time of sample collection was assigned using a two-step process. First, we used a conservative age range to assign menopausal status. If a woman was less than 43 years of age at the time of sample collection, she was designated premenopausal ($N = 218$); if she was older than 55 years, she was designated postmenopausal ($N = 139$). For women in the intermediate age group (43–55 years old), FSH (follicular stimulating hormone) levels were measured and used to assign menopausal status primarily based upon the manufacturer's test guidelines (premenopausal cutoff: FSH <=15 mIU/ml, postmenopausal cutoff: FSH >=23 mIU/ml) (*20*). Of those in the intermediate age range and assigned by FSH values ($N = 152$), 75 were designated premenopausal, 60 were postmenopausal, and the others were in between the two cutoff values and were designated perimenopausal ($N = 17$). Due to the paucity of samples collected from perimenopausal women, they were excluded. The final data set included 492 samples collected from 412 women (293 premenopausal and 199 postmenopausal samples). Of the 80 subjects with two separate samples, 63 had samples that were both collected prior to menopause, 6 subjects had samples that were both collected after menopause, and 11 had one sample collected before and one after menopause.

All blood samples were obtained with informed consent, and the Human Investigation Committee of the Radiation Effects Research Foundation (RERF) approved the study protocol.

Sample Storage and Serum Assays

Sera were collected in the Hiroshima and Nagasaki laboratories between 1969 and 1992 and stored in vials at −80°C. Samples for this study were pulled from the freezers simultaneously and assayed for total E_2, bioavailable E_2, testosterone, progesterone, prolactin, IGF-1, IGFBP-3, ferritin, and total protein and protein fractions (albumin and globulin). All assays were performed blinded to age, city of collection and radiation exposure but were not performed in duplicate due to the limited serum amounts available. Commercial laboratories with internal quality assurance systems performed all assays. Supplementary Table 1 (http:dx.doi.org/10.1667/RR2631.1.S1) lists the assays and their precision as reported by the kits' manufacturers.

Serum levels of IGF-1, IGFBP-3, FSH, prolactin, testosterone, progesterone and ferritin were measured at a single laboratory (SRL Laboratory, Hachioji, Japan). Total protein concentration and protein fractions were measured by SRL using the Biuret method and cellulose acetate membrane electrophoresis, respectively. Serum levels of total E_2 and percentage bioavailable E_2 were measured at a single laboratory (Mitsubishi Kagaku Bio-clinical Laboratories, Tokyo, Japan). Percentage bioavailable E_2 (the fraction of serum E_2 that is free or loosely bound to albumin) was determined by ammonium sulfate precipitation of SHBG-bound E_2 (*21*). Total E_2 was multiplied by this percentage to obtain the concentration of bioavailable E_2. Serum ferritin was measured by the conventional chemiluminescence enzyme immunoassay (CLEIA). When the aliquots of samples were transported to outside laboratories for testing, they were packed in appropriate protective shipping boxes with sufficient amounts of dry ice and checked at the time of receipt by the measuring laboratories.

Methods of Analysis

Due to the skewed distributions of the markers, analyses were performed using log-transformed values for all of the markers except IGF1 and IGFBP-3, which did not require transformation. Effects of radiation exposure were estimated with adjustment only for other factors that were significantly related to marker level for statistical efficiency. Explanatory factors considered were: age at time of sample collection, year of birth, menopausal status (pre or post), total protein, body mass index [weight (kg)/height (m^2)] and its square, history of exogenous hormone use (yes or no), age at time of radiation exposure (including dichotomy at 15 years as a surrogate for pre- or postmenarche at time of exposure), number of full-term pregnancies, age at first full-term delivery, and radiation dose. Decisions for inclusion of factors other than radiation dose were based on a backward selection process where the least significant explanatory factor was removed until all factors were significant at a level of $P \leq 0.05$. The variable selection process was conducted separately for each marker. Analyses were performed using a regression method for correlated (repeated) measurements (generalized estimating equations; GEE) (*22*). *P* values and 95% confidence intervals were based on the Wald statistic.

TABLE 1
Characteristics of Study Cohort and Collected Samples by Menopausal Status

Variable	Premenopausal samples (N = 293)	Postmenopausal samples (N = 199)	P value[a]
City (Hiroshima/Nagasaki)[b]	187/106	145/54	0.036
	Mean (range)	Mean (range)	
Age ATB (years)	13.4 (1.0–24.0)	28.2 (12.0–49.0)	<0.001
Age at collection (years)	40.3 (26.3–51.9)	57.7 (43.0–78.9)	<0.001
Radiation dose (weighted gray)	0.54 (0.0–3.2)	0.49 (0.0–2.8)	0.472
BMI (kg/m^2)	22.4 (15.6–32.4)	22.8 (14.6–39.3)	0.267
Number of full-term pregnancies	2.7 (0–11)	3.6 (0–11)	<0.001
Year of sample collection	1971.9 (1969–1991)	1975.4 (1970–1992)	<0.001
IGF-1 (ng/ml)	116.0 (0.0–320.0)	94.6 (62–130)	<0.001
IGFBP-3 (μg/ml)	2.53 (0.4–4.3)	2.37 (2.1–2.8)	0.002
	Median (interquartile range[c])	Median (interquartile range[c])	
Total E_2 (pg/ml)	86.65 (59.2–116.0)	18.50 (13.1–25.2)	<0.001
Bioavailable E_2 (pg/ml)	39.48 (27.3–53.7)	9.19 (5.8–13.2)	<0.001
Testosterone (ng/dl)	15.7 (10.4–23.3)	10.5 (6.7–18.3)	<0.001
Progesterone (ng/ml)	1.60 (0.40–10.0)	0.25 (0.19–0.30)	<0.001
Prolactin (ng/ml)	6.20 (4.0–9.2)	3.60 (2.4–5.1)	<0.001
Ferritin (ng/ml)	21.50 (7.5–41.0)	50.00 (29–94)	<0.001

Notes. The upper section is the count of samples by city. The middle section shows the averages and ranges of non-transformed variables. The bottom section shows the median and interquartile ranges of natural log-transformed variables.

[a] Counts tested with χ^2 test for independence, means tested with t test for equality, medians tested with the Wilcoxon rank-sum test for independent samples.

[b] 412 subjects (Hiroshima, N = 267/Nagasaki, N = 145). 80 subjects had multiple samples (63 subjects with two premenopausal samples, 6 subjects with two postmenopausal samples, 11 subjects with one premenopausal sample and one postmenopausal sample).

[c] 25th percentile to the 75th percentile.

Radiation effects were assessed using linear dose–response functions. Dose responses were estimated with and without regard to effect modification by menopausal status at the time of blood collection and ionizing radiation exposure before or after age 15. For markers that were transformed prior to analyses, the radiation effects were reported on the untransformed scale by exponentiating the regression coefficient obtained using the log-transformed data and represent the association between 1 Gy radiation dose and the percentage change in geometric mean of the marker level. IGF1 and IGFBP-3, which were not transformed, were also reported on a relative scale by dividing the dose coefficient by the regression constant. To test the strength of the observed radiation effects, we identified and removed the most influential observations using the size-adjusted criterion 2/sqrt(n) for the t-statistic-like standardized deletion diagnostic DFBETAS of Belsley *et al.* (*23*). To alleviate concerns about misclassification of menopausal status at the time of sample collection due to possible nonspecificity of the FSH results, we conducted a sub-analysis after restricting samples to those collected from women at ages less than 43 or greater than 55 (135 samples omitted) and compared results to the full analyses.

Statistical comparisons between samples collected pre- and postmenopause were performed using χ^2 tests (for counts), t tests for equality (for means) and Wilcoxon rank-sum tests for independent samples (for medians). Interactions of radiation effects by menopausal status or exposure before or after age 15 were tested using the likelihood ratio. To compare fits of statistically significant non-nested interaction models, the quasi-likelihood/independence criterion (QIC) was used (*24*). All results were obtained using Stata (*25*).

RESULTS

Table 1 shows the demographics of the study cohort and marker values for samples separated by menopausal status at the time of collection. The distribution of the radiation dose is broader than what would be expected from a random sampling of all cancer-free women since they were counter-matched (low, medium, high) against the dose status of the case to ensure a broad representation of doses. The average year of sample collection is somewhat later for postmenopausal samples because the AHS study is a fixed cohort; more women were postmenopausal in later calendar years.

Table 2 shows the agreement of assays for the 63 pairs of samples collected from women who were premenopausal at each collection. Comparisons of multiple samples collected from the same women while postmenopausal were not reported (N = 6 pairs). The earlier sample was collected at average age 39.7 years and the average interval between sample collections was 2.8 years. Correlation coefficients ranged from 0.11 for progesterone to 0.75 for ferritin. A paired t test comparing the difference of the paired values with zero was not significant for any marker except for serum prolactin, where the earlier value proved to be significantly higher than the second value.

The first column of Table 3 shows the associations of radiation with marker levels with no allowance for effect modification but with adjustment for other significant factors. No significant radiation associations were observed in any of the markers when ignoring menopausal status. Columns two through five in Table 3 show the radiation associations separately by menopausal status at the time of sample collection or by age

TABLE 2
Comparison of Assay Values from Women Donating Multiple Samples

Assayed biomarker	*N*	Sample 1 average value (SD)	Sample 2 average value (SD)	Correlation	Sample 1 − Sample 2 average value (SD)	*P* value
Log total E_2 (pg/ml)	63	4.47 (1.02)	4.34 (0.54)	0.14	0.13 (1.10)	0.10
Log bioavailable E_2 (pg/ml)	63	3.65 (0.94)	3.64 (0.54)	0.16	0.014 (1.03)	0.42
Log testosterone (ng/dl)	57	2.74 (0.59)	2.74 (0.59)	0.52	0.0066 (0.54)	0.55
Log progesterone (ng/ml)	54	0.49 (1.71)	0.53 (1.64)	0.11	−0.042 (2.3)	0.54
Log prolactin (ng/ml)	57	1.86 (0.85)	1.47 (0.61)	0.28	0.39 (0.91)	0.001
Log ferritin (ng/ml)	56	3.12 (1.30)	3.11 (1.35)	0.75	0.0082 (0.83)	0.93
IGF-1 (ng/ml)	57	129.0 (69.7)	149 (67.9)	0.16	−20.3 (88.1)	0.19
IGFBP-3 (μg/ml)	57	2.51 (0.55)	2.54 (0.43)	0.36	−0.029 (0.60)	0.96

Notes. Samples are confined to multiple premenopausal samples (there were only six persons who donated multiple postmenopausal samples). The average age at the time of Sample 1 collection was 37.9 years and the average year of collection was 1970.9. The average age at the time of collection for the second sample was 40.8 years and the average year of collection was 1973.7. Values in parentheses (SD) after averages are standard deviations. The *P* value in the last column is a paired *t* test (H_o: Sample 1 – Sample 2 = 0).

at time of bombing (≤15 or >15 years; ATB15). Because menopausal status at blood donation was highly correlated with ATB15 in this study sample (particularly for a woman's first/only serum sample), the latter two columns largely mirror the results displayed in the former two. Total and bioavailable E_2, testosterone and IGF-1 demonstrated statistically significant associations with radiation dose in one or both periods.

Total E_2 (Fig. 1) decreased with radiation dose in the premenopausal period (relative change 0.89 at 1 Gy; 95% CI: 0.80, 0.99; $P = 0.027$), or in other words, the level of total E_2 changed as $k*0.89^{dose}$, where k is the level of total E_2 when the dose was zero (results from other log-transformed variables are interpreted in the same way). The level of total E_2 increased with dose in the postmenopausal period (relative change 1.17 at 1 Gy; 95% CI: 1.01, 1.36; $P = 0.037$). Effect modification by menopausal status was highly significant ($P = 0.003$) and remained significant after removing the most influential values ($P = 0.038$). The association of total E_2 with radiation dose was similar when categorizing subjects based on ATB15 (rather than menopausal status at the time of collection); relative change for a 1-Gy dose at age less than 15 years was 0.86 (95% CI: 0.76, 0.96; $P = 0.010$) and relative change for exposure after age 15 was 1.12 (95% CI: 0.99, 1.26; $P = 0.063$). Models used to estimate the radiation effects on total E_2 included menopausal status, age at sample collection, total protein, BMI and a BMI*postmenopause interaction. The QIC values for the two models were very similar, but the value for the ATB15 interaction model was slightly lower (i.e. better fit) than for the model with interaction by menopausal status.

Bioavailable E_2 (Fig. 2) showed a significant decrease with radiation dose in the premenopausal period, with a relative change of 0.88 at 1 Gy (95% CI: 0.80, 0.98; $P = 0.022$) and a significant increase with radiation dose in the postmenopausal period with a relative change of 1.21 at 1 Gy (95% CI: 1.04, 1.40; $P = 0.011$). Effect modification by menopausal status was highly significant ($P < 0.001$) and remained so after exclusion of influential observations ($P = 0.003$). As with total E_2, bioavailable E_2 also demonstrated similar radiation effects according to whether exposure was before or after age 15 (0.87 at 1 Gy; 95% CI: 0.77, 0.98; $P = 0.019$ and a relative increase of 1.13 at 1 Gy; 95% CI: 1.00, 1.27; $P = 0.045$, respectively). The model used to estimate the radiation effects on bioavailable E_2 included menopause status, age at sample collection and total protein. Again the QIC value was slightly lower for the model with ATB15 interaction.

Testosterone (Fig. 3) exhibited an increase with radiation dose in the postmenopausal period (relative change 1.30 at 1 Gy; 95% CI: 1.13, 1.49; $P < 0.001$) and a marginally significant decrease with radiation dose in the premenopausal period (0.90 at 1 Gy; 95% CI: 0.81, 1.00; $P = 0.049$). Estimates of effect modification by menopausal status were highly significant when estimates were derived using all observations ($P < 0.001$) and after excluding outliers ($P = 0.003$), whereas no effect modification was evident with ATB15. The testosterone model included menopause status, age at sample collection, total protein and a total protein*postmenopause interaction, and BMI.

IGF-1 (Fig. 4) displayed a significant increase with radiation dose in the premenopausal period (relative change 1.11 per Gy; 95% CI: 1.02, 1.18; $P = 0.024$). Since the values from this assay were not log-transformed, the results are interpreted as the percentage change in marker levels per gray. No significant change with dose was observed in the postmenopausal period (relative change 0.91 per Gy; 95% CI: 0.75, 1.04; $P = 0.18$). Similar effects were seen when categorizing the women by age at exposure. Effect modification by menopausal status was significant ($P = 0.014$) and remained so after removing the most influential observations ($P = 0.017$). Comparing QIC values indicated that the model with menopausal interaction model was preferable to the one with ATB15 interaction. Estimates of the radiation effects were adjusted for age at sample collection, year of birth, total protein*premenopause interaction, and number of full-term pregnancies.

TABLE 3
Relative Radiation Associations with Adjusted Mean Marker Values and Effect Modification By Menopausal Status and Age at Radiation Dose

Marker	Relative radiation association at 1 Gy no effect modification (95% confidence intervals), *P* value	Relative radiation association at 1 Gy Separately by effect-modifier category[a] (95% confidence intervals), *P* value			
		Menopause status at time of sample collection		Age at time of the bomb	
		Pre (N = 293)	Post (N = 199)	≤15 (N = 190)	>15 (N = 302)
Total E_2[b,d]	0.97	0.89	1.17	0.86	1.12
	(0.89, 1.06)	(0.80, 0.99)	(1.01, 1.36)	(0.76, 0.96)	(0.99, 1.26)
	P = 0.52	**P = 0.027**	**P = 0.037**	**P = 0.010**	P = 0.063
		Interaction **P = 0.003**		Interaction **P = 0.002***	
Bioavailable E_2[b,e]	0.98	0.88	1.21	0.87	1.13
	(0.93, 1.06)	(0.80, 0.98)	(1.04, 1.40)	(0.77, 0.98)	(1.00, 1.27)
	P = 0.84	**P = 0.022**	**P = 0.011**	**P = 0.019**	**P = 0.045**
		Interaction **P <0.001**		Interaction **P = 0.002***	
Testosterone[b,f]	1.02	0.90	1.30	0.97	1.06
	(0.93, 1.12)	(0.81, 1.00)	(1.13, 1.49)	(0.84, 1.10)	(0.93, 1.20)
	P = 0.70	**P = 0.049**	**P < 0.001**	P = 0.617	P = 0.387
		Interaction **P < 0.001**		Interaction P = 0.337	
Progesterone[b,g]	1.08	1.08	1.10	1.00	1.21
	(0.90, 1.30)	(0.86, 1.35)	(0.81, 1.50)	(0.77, 1.29)	(0.93, 1.58)
	P = 0.39	P = 0.52	P = 0.54	P = 0.992	P = 0.16
		Interaction P = 0.905		Interaction P = 0.313	
Prolactin[b,h]	1.01	0.97	1.07	0.94	1.08
	(0.91, 1.11)	(0.86, 1.09)	(0.91, 1.26)	(0.82, 1.08)	(0.95, 1.24)
	P = 0.905	P = 0.641	P = 0.384	P = 0.413	P = 0.230
		Interaction P = 0.313		Interaction P = 0.161	
IGF-1[c,i]	1.04	1.11	0.91	1.11	0.98
	(0.96, 1.10)	(1.02, 1.18)	(0.75, 1.04)	(0.99, 1.20)	(0.85, 1.07)
	P = 0.29	**P = 0.024**	P = 0.18	P = 0.061	P = 0.850
		Interaction **P = 0.014***		Interaction **P = 0.091**	
IGFBP-3[c,j]	1	1.01	0.98	1.00	0.93
	(0.97, 1.03)	(0.97, 1.05)	(0.92, 1.05)	(0.97, 1.04)	(0.97, 1.03)
	P = 0.89	P = 0.48	P = 0.44	P = 0.81	P = 0.82
		Interaction P = 0.28		Interaction P = 0.98	
Ferritin[b,k]	0.97	0.98	0.96	0.87	1.07
	(0.83, 1.13)	(0.81, 1.18)	(0.73, 1.27)	(0.70, 1.09)	(0.86, 1.32)
	P = 0.72	P = 0.81	P = 0.78	P = 0.22	P = 0.55
		Interaction P = 0.92		Interaction P = 0.19	

Notes. Numbers in parentheses are 95% confidence intervals. The first column shows the radiation associations without adjustment for menopause status at the time of sample collection or age at the time of the exposure from the atomic bombings. The right two sets of columns show the radiation associations by menopause status (columns 2, 3) and age less than or greater than 15 at the time of the bombing (columns 4, 5). The "Interaction *P*" values test whether the results differ significantly by menopausal status or age at the time of the bombing. Note that there were no detected effects when effect modification was ignored. The models were developed using a backwards elimination process for statistical efficiency as described in the text. Covariates for each model are listed in the footnotes. All effect modification models included the main effect variable regardless of whether it was selected in the elimination process. *QIC value indicates that this interaction model was better than the alternative interaction model. This score was compared only between the two interaction models and only when both models were significant.

[a] Effects of radiation are displayed separately for each of two categories of either menopausal status at time of serum collection (pre- or postmenopause) or age at time of exposure (up to age 15 or after age 15). The main effect was always included when testing the interaction.

[b] Log-transformed variable (relative change is calculated using X^{dose}, where X is the reported coefficient).

[c] Non-transformed variable (relative change is calculated using X * dose, where X is the reported coefficient).

[d] Adjusted for age and menopause status at sample collection, total protein, BMI and BMI*postmenopause interaction.

[e] Adjusted for age and menopause status at sample collection, total protein.

[f] Adjusted for age and menopause status at sample collection, total protein, total protein*postmenopause interaction, BMI.

[g] Adjusted for menopause status at sample collection, total protein.

[h] Adjusted for age and menopause status at sample collection.

[i] Adjusted for age at sample collection, year of birth, total protein*premenopause interaction, number of full-term pregnancies.

[j] Adjusted for year of birth, total protein*premenopause interaction, BMI, BMI^2.

[k] Adjusted for age and menopausal status at sample collection, BMI, number of full-term pregnancies.

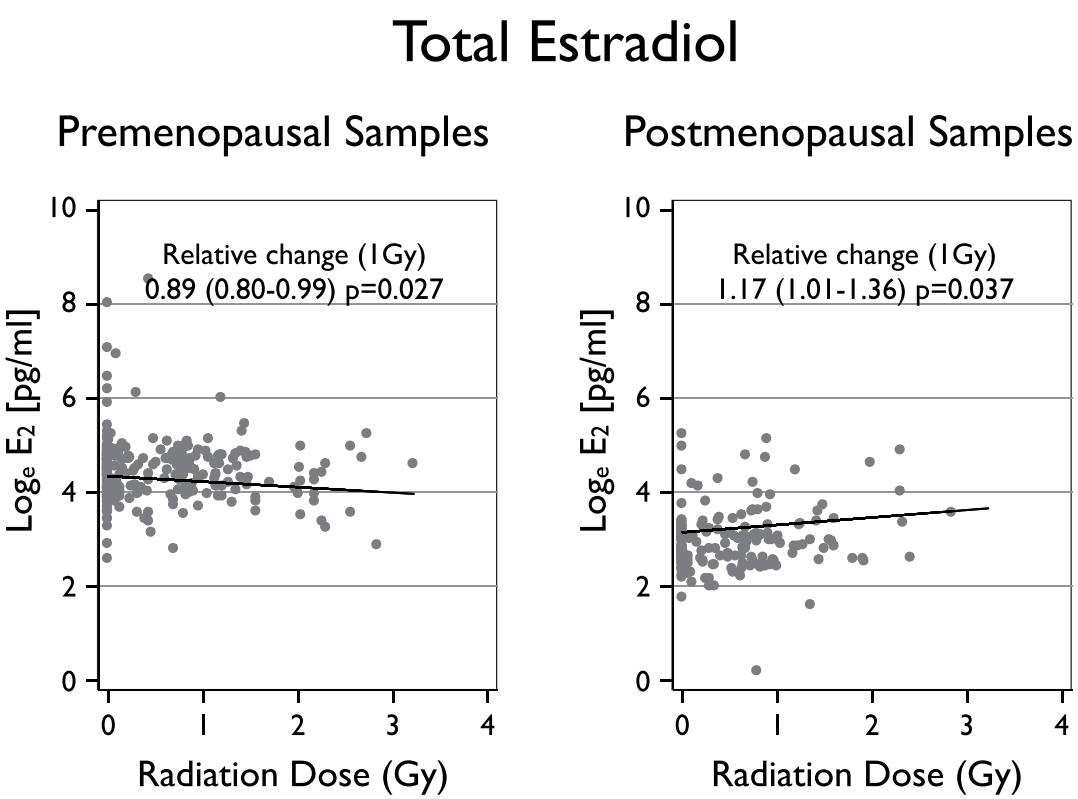

FIG. 1. Log total estradiol (E_2) by menopausal status and radiation dose. The relative change is the percentage change at a dose of 1 Gy. The regression lines and *P* values are based on the final models obtained for the markers (Table 3). The slopes of the two regression lines by menopausal status are significantly different ($P = 0.003$).

The other markers (IGFBP-3, progesterone, prolactin and ferritin) demonstrated no associations with radiation dose, either with or without effect modification. No effect modification of the radiation effects was observed in relation to the sample storage period, time since exposure, or the number of full-term pregnancies, although E_2 levels did increase at a marginally significant level for each additional full-term pregnancy (results not shown). Model fits generally improved when including the number of full-term pregnancies (as either an effect modifier or a main effect), but the estimated coefficients for the number of pregnancies were never statistically significant and therefore the results were not reported. No improvements in model fit were detected by the addition of a quadratic dose term. Point estimates were generally unchanged by the removal of samples retrieved when women were aged 43 to 55.

DISCUSSION

The present study was designed to investigate the associations of ionizing radiation exposure with sex and growth hormones in cancer-free A-bomb survivors using stored sera. Increasing levels of ionizing radiation exposure led to disparate changes by menopausal status in a number of serum markers. Levels of E_2 and bioavailable E_2 increased in postmenopausal samples while they decreased in premenopausal samples. Caution must be used when interpreting the results in premenopausal samples, especially E_2 levels, which are highly variable in premenopausal women due to the menstrual cycle. Table 2 illustrates the high variability of serum marker levels among premenopausal women. Controlling for the day of the menstrual cycle was not possible, because the clinical examination protocol did not attempt to record these values. It should be noted, however, that the point estimates for the radiation findings were largely unchanged after separately removing the most influential measurements (e.g., those associated with the E_2 peak at ovulation) and excluding those in the perimenopausal age range 43–55. Also, it is unlikely that there is any association with the timing of blood sample collection and radiation dose, eliminating most concerns of confounding. Nevertheless, chance findings for the premenopausal hormone associations cannot be completely ruled out.

The magnitude of change in total E_2 levels was about 10% at 1 Gy of radiation (negative in premenopausal women and positive in postmenopausal women). It is not clear that either of these are clinically significant changes, particularly among premenopausal women where there is so much variation. However, if E_2 levels were assigned to quartiles in samples taken from postmenopausal women, a 10% change in any particular result would generally be sufficient to increase its quartile assignment by one

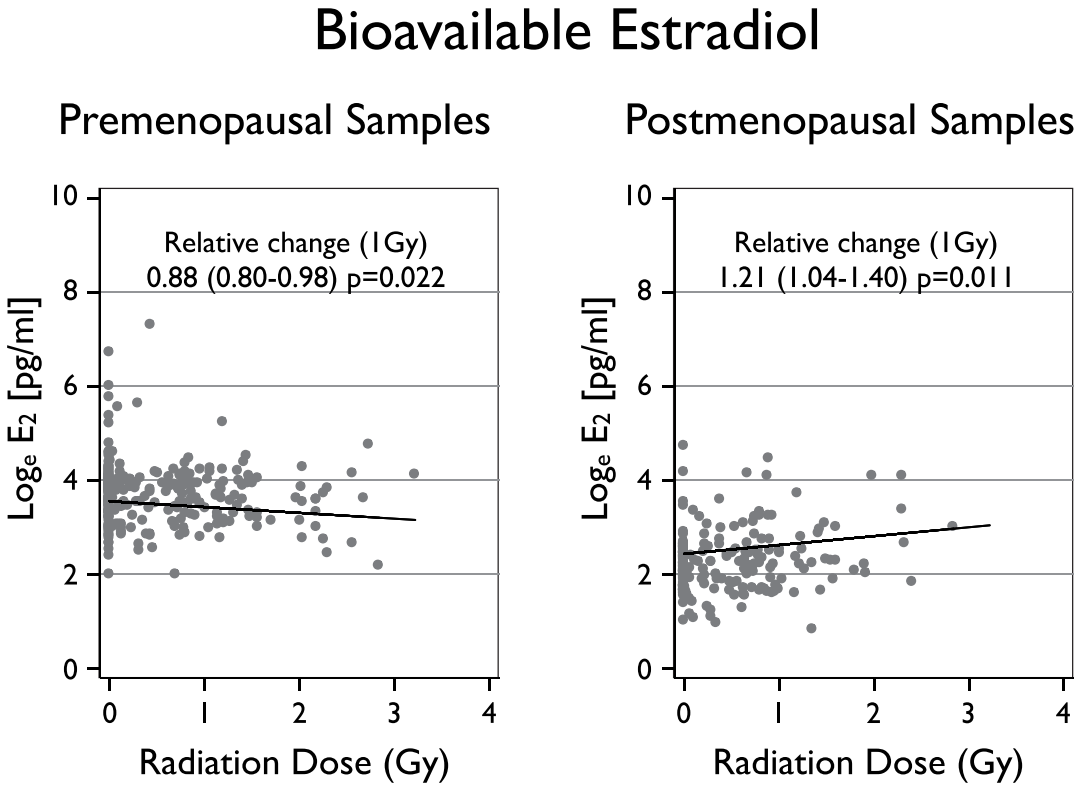

FIG. 2. Log bioavailable estradiol (E_2) concentration by menopausal status and radiation dose. The relative change is the percentage change at a dose of 1 Gy. The regression lines and *P* values are based on the final models obtained for the markers (Table 3). The slopes of the two regression lines by menopausal status are significantly different ($P < 0.001$).

category. Interpolating the results from Fig. 1 of the meta-analysis performed by Key *et al.* (*7*), this increase in total E_2 would roughly correspond to a 25% increase in breast cancer risk.

The significance of IGF-1 and IGFBP-3 levels in pre- or postmenopausal breast cancer has been under debate. The most recent evidence indicates that high levels of IGF-1 confer an increased risk of breast cancer (*10*). In our study, radiation exposure appeared to increase the level of IGF-1 in premenopausal women but had no effect on levels of IGF-1 in postmenopausal women. Again, however, large variations were observed in the premenopausal IGF-1 levels. IGFBP-3 was not associated with radiation exposure.

If the results are correct, questions arise regarding the radiation-related biological pathways that may affect sex hormone levels, particularly the most potent hormone E_2. In premenopausal women, E_2 is produced primarily by the ovaries whereas after ovarian involution, estrogen is primarily produced in adipose tissue by converting androgen to estrogen via aromatase. A number of speculative mechanisms for an association between radiation and sex steroid alterations can be suggested, including damage to primordial ovarian follicles leading to decreased ovarian aromatase and depressed E_2 production as observed in the samples taken from women who were exposed at less than 15 years of age (*26, 27*). Ataya *et al.* reported similar findings with decreased ovarian size and long-term decreased serum E_2 levels in rhesus monkeys with ovarian exposure to ionizing radiation (*28*). Larsen *et al.* reported decreased ovarian volume in spontaneously ovulating women who had received chemotherapy and radiotherapy for cancers diagnosed at mean age 5.4 years; however, E_2 levels were reported to be higher in those receiving (any type of) therapy compared to a control group of similar age (*29*).

For the results seen among older birth cohorts (i.e. postmenopausal samples), radiation-induced upregulation of inflammatory cytokines, specifically TNFα, can lead to increased estrogen biosynthesis (*30–33*); radiation can also alter lipid metabolism (*34–36*), possibly leading to hyperandrogenism through hypothalamus-pituitary-adrenal axis dysregulation (*37*). Either of these latter mechanisms would be consistent with the observed increased E_2 and testosterone levels among the postmenopausal women.

No effect modification by the number of full-term pregnancies was evident in the radiation effects on any of the markers. The number of women reporting any history of exogenous hormone intake was less than 10%, and that variable was not a significant risk factor in any of the tested models. Therefore, we do not believe that hormone treatment had a meaningful impact on our

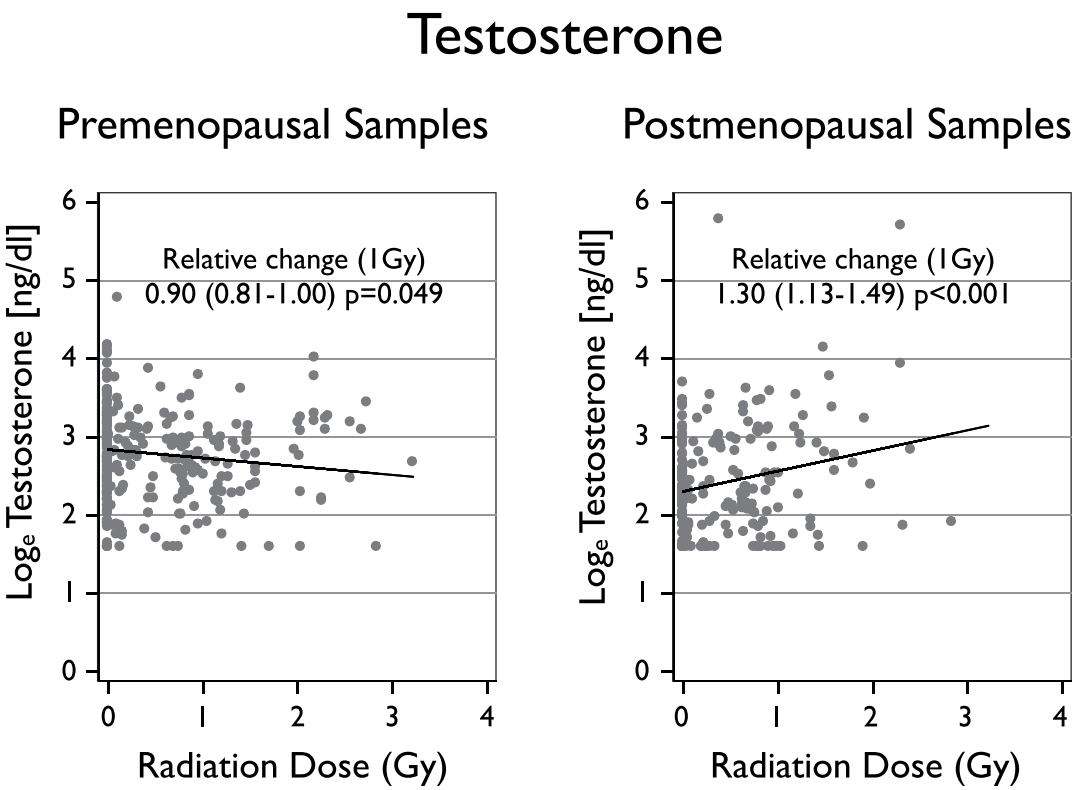

FIG. 3. Log testosterone by menopausal status and radiation dose. The relative change is the percentage change at a dose of 1 Gy. The regression lines and *P* values are based on the final models obtained for the markers (Table 3). The slopes of the two regression lines by menopausal status are significantly different ($P < 0.001$).

findings. Interaction models categorizing exposures before or after age 15 more often had a better fit than models categorized by menopausal status at the time of serum collection. However, this may be due to misclassification of menopausal status as opposed to an important biological effect involving menarche status at the time of radiation exposure. McDougall *et al.* found no association of breast cancer risk and menarche status at the time of radiation exposure but did not consider serum marker levels (*38*).

Associations of radiation and markers modified by menopausal status were tested in the results of eight bioassays (the two parallel analyses of modification by either menopause status or ATB15 are effectively the same), which may raise concerns of finding associations due to multiple testing. Using the conservative Bonferroni correction for multiple testing ($\alpha = 0.05/8 = 0.00625$), all of the tests for interaction remain significant with the exception of IGF-1 ($P = 0.014$).

The study boasts a number of strengths, including well-characterized radiation doses to a representative population of women, and data collected prospectively from cancer-free women as part of a long-term follow-up regimen, including medical history data on hormone-related conditions. However, there are also several weaknesses. The day of blood collection in relation to the menstrual cycle was not available, which is problematic for premenopausal estradiol measurements in particular. The use of FSH to assign menopausal status has been shown to be not fully specific (*39*). It is also likely that misclassification occurred when assigning menarche status by age. Due to the limited number of samples, duplicate assays for quality control were not attempted. A limited number of women did have multiple samples collected while premenopausal, for which the correlation between marker assays was generally poor, probably due to different times in the menstrual cycle. Despite these limitations, no major changes in the interactions between the measured radiation associations and menopausal status were detected after separately removing the most influential observations and those observations drawn from women in the perimenopausal age range, which helps confirm the robustness of the associations.

Conclusions

Previous studies have proven the potency of ionizing radiation as a carcinogen via direct damage effects to DNA while separate lines of research have established serum levels of E_2, testosterone and IGF1 as risk factors for breast cancer. The results of this study suggest that ionizing radiation may lead to changes in serum levels of cancer-related hormones and proteins in cancer-free women and that these changes are dependent upon

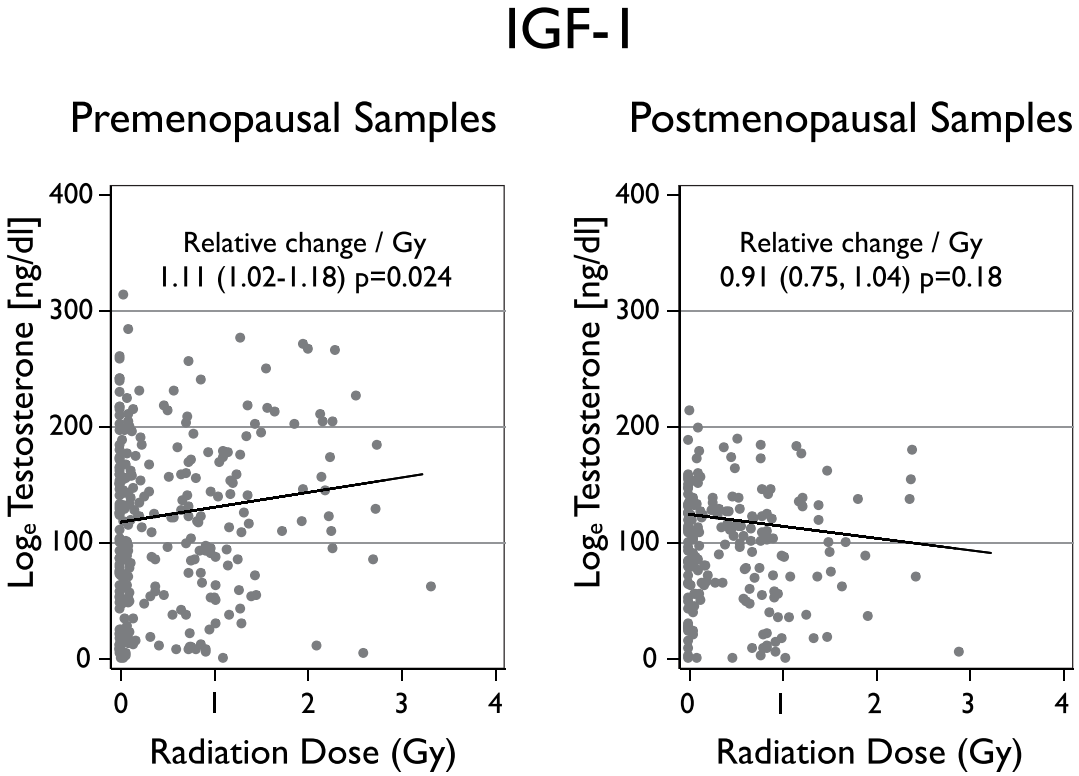

FIG. 4. IGF-1 by menopausal status and radiation dose. The relative change is the percentage change at a dose of 1 Gy. The regression lines and *P* values are based on the final models obtained for the markers (Table 3). The slopes of the two regression lines by menopausal status are significantly different ($P = 0.014$).

either the menopausal status at the time of collection or the menarche status at the time of exposure. Several mechanisms for these changes are suggested, but they are all speculative. Researchers of cancer etiology after whole-body ionizing radiation exposure should be aware of possible underlying changes in the levels of serum markers, which may confer independent carcinogenic risks.

ACKNOWLEDGMENTS AND FUNDING

We deeply thank the participating members of the AHS. The Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan is a private, non-profit foundation funded by the Japanese Ministry of Health, Labor and Welfare (MHLW) and the U.S. Department of Energy (DOE), the latter in part through the National Academy of Sciences. This publication was supported by RERF Research Protocol RP 06-02 and by US National Cancer Institute contract NO1-CP-31012 and the Japanese Ministry of Education, Culture, Sports, Science and Technology grants 14031227 and 15026220.

Received: March 27, 2011; accepted: May 24, 2011; published online: June 30, 2011

REFERENCES

1. Brenner DJ, Hall EJ. Computed tomography – an increasing source of radiation exposure. N Engl J Med 2007; 357:2277–84.
2. Preston DL, Mattsson A, Holmberg E, Shore R, Hildreth NG, Boice JD. Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. Radiat Res 2002: 158:220–35.
3. Preston DL, Ron E, Tokuoka S, Funamoto S, Nishi N, Soda M, et al. Solid cancer incidence in atomic bomb survivors: 1958–1998. Radiat Res 2007; 168:1–64.
4. UNSCEAR. Sources and effects of ionizing radiation. United Nations Scientific Committee on the Effects of Atomic Radiation: UNSCEAR 2000 Report to the General Assembly, with scientific annexes; 2000.
5. Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998; 90:1292–9.
6. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Natl Cancer Inst 1995; 87:190–7.
7. Endogenous Hormones and Breast Cancer Collaborative Group, Key T, Appleby PN, Reeves GK, Roddam AW. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002; 94:606–16.
8. Henderson BE, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis 2000; 21:427–33.
9. Russo J, Russo IH. Genotoxicity of steroidal estrogens. Trends Endocrinol Metab 2004; 15:211–4.
10. Endogenous Hormones and Breast Cancer Collaborative Group, Key T, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010; 11:530–42.
11. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004; 363:1346–53.
12. Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, et al. Serum sex steroids in premenopausal women and breast cancer

risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2005; 97:755–65.

13. Tworoger SS, Eliassen AH, Sluss P, Hankinson SE. A prospective study of plasma prolactin concentrations and risk of premenopausal and postmenopausal breast cancer. J Clin Oncol 2007; 25:1482–8.
14. Russo J, Hu YF, Silva ID, Russo IH. Cancer risk related to mammary gland structure and development. Microsc Res Tech 2001; 52:204–23.
15. Yamada M, Wong FL, Fujiwara S, Akahoshi M, Suzuki G. Noncancer disease incidence in atomic bomb survivors, 1958–1998. Radiat Res 2004; 161:622–32.
16. (Young RW, Kerr GD, editors. Reassessment of the atomic bomb radiation dosimetry for Hiroshima and Nagasaki–Dosimetry System 2002. Hiroshima, Japan: Radiation Effects Research Foundation; 2005. 998 p.
17. Cologne JB, Sharp GB, Neriishi K, Verkasalo PK, Land C, Nakachi K. Improving the efficiency of nested case-control studies of interaction by selecting controls using counter matching on exposure. Int J Epidemiol 2004; 33:485–92.
18. Biesheuvel CJ, Vergouwe Y, Oudega R, Hoes AW, Grobbee DE, Moons KG. Advantages of the nested case-control design in diagnostic research. BMC Med Res Methodol 2008; 8:48.
19. Pierce DA, Stram DO, Vaeth M. Allowing for random errors in radiation dose estimates for the atomic bomb survivor data. Radiat Res 1990; 123:275–84.
20. Tokyo Special Reference Laboratories (SRL), Inc [Internet]. 卵胞刺激ホルモン (FSH) 基準値 [Follicle-stimulating hormone (FSH) reference levels]. [cited 2010 Jun 7]. Available from: http://www.srl.info/srlinfo/news/2003-14.htm. Japanese.
21. Pearce S, Dowsett M, Jeffcoate SL. Three methods compared for estimating the fraction of testosterone and estradiol not bound to sex-hormone-binding globulin. Clin Chem 1989; 35:632–5.
22. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986; 73:13–22.
23. Belsley DA, Kuh E, Welsch RE. Regression diagnostics: identifying influential data and sources of collinearity. New York: Wiley; 1980.
24. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics 2001; 57:120–5.
25. StataCorp. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP; 2009.
26. Lee CJ, Yoon YD. Gamma-radiation-induced follicular degeneration in the prepubertal mouse ovary. Mutat Res 2005; 578: 247–55.
27. Lee YK, Chang HH, Kim WR, Kim JK, Yoon YD. Effects of gamma-radiation on ovarian follicles. Arhiv za higijenu rada i toksikologiju 1998; 49:147–53.
28. Ataya K, Pydyn E, Ramahi-Ataya A, Orton CG. Is radiation-induced ovarian failure in rhesus monkeys preventable by luteinizing hormone-releasing hormone agonists?: Preliminary observations. J Clin Endocrinol Metab 1995; 80:790–5.
29. Larsen EC, Müller J, Schmiegelow K, Rechnitzer C, Andersen AN. Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. J Clin Endocrinol Metab 2003; 88:5307–14.
30. Frasor J, Weaver A, Pradhan M, Mehta K. Synergistic up-regulation of prostaglandin E synthase expression in breast cancer cells by 17β-estradiol and proinflammatory cytokines. Endocrinology 2008; 149:6272–9.
31. Salama S, Kamel M, Diaz-Arrastia C, Xu X, Veenstra TD, Salih S, et al. Effect of tumor necrosis factor on estrogen metabolism and endometrial cells: potential physiological and pathological relevance. J Clin Endocrinol Metab 2008; 94:28–93.
32. Hayashi T, Morishita Y, Kubo Y, Kusunoki Y, Hayashi I, Kasagi F, et al. Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. Am J Med 2005; 118:83–6.
33. Nakachi K, Hayashi T, Imai K, Kusunoki Y. Perspectives on cancer immuno-epidemiology. Cancer Sci 2004; 95:921–9.
34. Bezemer ID, Rinaldi S, Dossus L, Gils CH, Peeters PH, Noord PA, et al. C-peptide, IGF-I, sex-steroid hormones and adiposity: a cross-sectional study in healthy women within the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Causes Control 2005; 16:561–72.
35. Akahoshi M, Amasaki Y, Soda M, Hida A, Imaizumi M, Nakashima E, et al. Effects of radiation on fatty liver and metabolic coronary risk factors among atomic bomb survivors in Nagasaki. Hypertens Res 2003; 26:965–70.
36. Wong FL, Yamada M, Sasaki H, Kodama K, Hosoda Y. Effects of radiation on the longitudinal trends of total serum cholesterol levels in the atomic bomb survivors. Radiat Res 1999; 151:736–46.
37. Pasquali R. Obesity and androgens: facts and perspectives. Fertil Steril 2006; 85:1319–40.
38. McDougall JA, Sakata R, Sugiyama H, Grant E, Davis S, Nishi N, et al. Timing of menarche and first birth in relation to risk of breast cancer in A-bomb survivors. Cancer Epidemiol Biomarkers Prev 2010; 19:1746–54.
39. Henrich JB, Hughes JP, Kaufman SC, Brody DJ, Curtin LR. Limitations of follicle-stimulating hormone in assessing menopause status: findings from the National Health and Nutrition Examination Survey (NHANES 1999–2000). Menopause 2006; 13:171–7.