



Published in final edited form as:

Int J Radiat Biol. 2018 February ; 94(2): 97–105. doi:10.1080/09553002.2018.1419303.

Serum bioavailable estradiol may alter radiation risk of postmenopausal breast cancer: a nested case-control study

J. Grant Eric^{a,*}, B. Cologne John^b, B. Sharp Gerald^c, Eguchi Hidetaka^d, G. Stevens Richard^e, Izumi Shizue^f, Kim Young-Min^g, González Amy Berrington de^h, Ohishi Wakaⁱ, and Nakachi Kei^j

^aRadiation Effects Research Foundation, Hiroshima, Japan

^bDepartment of Statistics, Radiation Effects Research Foundation, Hiroshima, Japan

^cEpidemiology Branch, Basic Sciences Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

^dTranslational Research Division, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan

^eDepartment of Community Medicine, University of Connecticut Health Center, Connecticut, USA

^fCenter for Data Science Education and Research, Shiga University, Hikone, Japan

^gDepartment of Statistics, College of Natural Science, Kyungpook National University, Daegu, South Korea

^hDivision of Cancer Epidemiology and Genetics, NCI/NIH, Bethesda, Maryland, USA

ⁱDepartment of Clinical Studies, Radiation Effects Research Foundation, Hiroshima, Japan

^jDepartment of Molecular Biosciences, Radiation Effects Research Foundation, Hiroshima, Japan

Abstract

Purpose: Ionizing radiation and high levels of circulating estradiol are known breast cancer carcinogens. We investigated the risk of first primary postmenopausal breast cancer in relation to the combined effects of whole-body ionizing radiation exposure and prediagnostic levels of postmenopausal sex hormones, particularly bioavailable estradiol (bE₂).

Materials and Methods: A nested case-control study of 57 incident breast cancer cases matched with 110 controls among atomic bomb survivors. Joint effects of breast radiation dose and circulating levels of sex hormones were assessed using binary regression and path analysis.

Results and Conclusions: Radiation exposure, higher levels of bE₂, testosterone and progesterone, and established reproductive risk factors were positively associated with postmenopausal breast cancer risk. A test for mediation of the effect of radiation via bE₂ level suggested a small (14%) but significant mediation (p=0.002). The estimated interaction between

*Eric J. Grant, Department of Epidemiology, Radiation Effects Research Foundation, Hijiyama Park 5-2, Minami-ku, Hiroshima 732-0815, Japan egrant@ref.or.jp Tel: +81-82-261-1937 Fax: +81-82-262-9768.

The authors report no conflicts of interest

radiation and bE_2 was large but not significant (interaction=3.86; $p=0.32$). There is accumulating evidence that ionizing radiation not only damages DNA but also alters other organ systems. While caution is needed, some portion of the radiation risk of postmenopausal breast cancer appeared to be mediated through bE_2 levels, which may be evidence for cancer risks due to both direct and indirect effects of radiation.

Keywords

breast cancer; hormones; estradiol; radiation; postmenopausal; interaction; mediation

Background

Evidence is accumulating that ionizing radiation not only damages DNA directly but also may act indirectly by altering immunologic, endocrinologic, and metabolic systems (Hayashi et al. 2005; Wong et al. 1999; Bezemer et al. 2005; Akahoshi et al. 2003). An unanswered fundamental question is whether observed systemic changes mediate part of the total effect, i.e., are biological factors in systems affected by radiation exposure helping to produce health endpoints typically associated solely with radiation effects?

Postmenopausal breast cancer has been associated with high levels of circulating estradiol. A number of factors can modulate a woman's cumulative exposure or breast sensitivity to estradiol. Those factors include age at menarche, age at menopause, age at first delivery, parity, breast feeding, and BMI (see, for example, Russo & Russo (I. H. Russo & J. Russo 2011)).

Exposure to ionizing radiation (IR) is another well-established risk factor for breast cancer. IR can directly damage DNA and is a risk factor for breast cancer in atomic bomb survivors (Land, Hayakawa, Machado, Y. Yamada, Pike, Akiba & Tokunaga 1994a), women exposed to therapeutic radiation (Carmichael et al. 2003) or high diagnostic radiation (Howe & McLaughlin 1996; Boice et al. 1991), excessive environmental radiation (Bauer et al. 2005; Ostroumova et al. 2008), and occupationally-exposed women (Sigurdson et al. 2003; Preston et al. 2010). Land et al. showed that reproductive-history risk-related factors associated with lower cumulative exposure to estradiol (parity, earlier age at first birth, cumulative duration of lactation) are associated with a lower radiation risk and were able to statistically reject an additive model in favor of a multiplicative model for the joint effect of radiation and reproductive factors (Land, Hayakawa, Machado, Y. Yamada, Pike, Akiba & Tokunaga 1994b). Land et al. did not, however, measure endogenous hormones directly. Kabuto et al (Kabuto et al. 2000) measured bioavailable estradiol (bE_2) and other hormones using stored sera, but were unable to see evidence of interaction between radiation and hormone levels using a log-linear model. The lower radiation risk among women with reduced exposure to endogenous estradiol coupled with lack of evidence for direct interaction between radiation and estradiol prompted us to examine in greater detail the joint effect of radiation and estradiol.

We recently found that postmenopausal serum hormone levels of bE_2 were positively associated with radiation dose in cancer-free atomic bomb survivors, which could be an example of systemic effects occurring after radiation exposure (Grant et al. 2011). This

finding raises the possibility that estradiol might act as a mediator of some proportion of the overall radiation risk of breast cancer. Therefore, our initial research question was whether the impact of radiation on the risk of postmenopausal breast cancer is mediated via raised levels of bE_2 (see Figure 1). A second question was whether Land et al's observation of multiplicative joint effects between reproductive-history factors and radiation exposure are also evident using postmenopausal levels of circulating sex hormones, primarily bioavailable estradiol (bE_2). Women of the atomic bomb survivor cohort provide a unique opportunity to study these questions in a prospective setting among humans.

Methods

Study Design, Selection, and Eligibility

A nested case control design was employed (Langholz 2005). Study subjects were women selected from the Radiation Effects Research Foundation's (RERF) Adult Health Study (AHS), a longitudinal cohort study with biennial clinical examinations of atomic bomb survivors initiated in 1958 (M. Yamada et al. 2004). Eligible women had stored sera, breast radiation dose estimates (Young & Kerr 2005), and were cancer-free for at least two years after serum collection (all sera were collected after menopause, see *Determining menopausal status*, below). Eligibility was further restricted to women who had no prior malignancies, were free from various hormone-related medical conditions, including oophorectomy, and did not have exogenous hormone treatment at the time of serum collection. The primary outcome was the diagnosis of a first-primary malignant breast cancer identified from the Hiroshima and Nagasaki cancer registries (Mabuchi et al. 1994).

Case selection

Originally, 171 first primary breast cancer cases (regardless of menopausal status) were identified. Samples could not be recovered for 19 cases and another 31 samples stored via freeze-drying were excluded due to unreliable reconstitution. Seventeen women who suffered a premenopausal breast cancer were excluded. Another 44 women did not have samples collected after menopause. Other cases were excluded for: no matching control (1), hormone treatment (1), and oophorectomy (1). The final dataset included 57 cases diagnosed with postmenopausal breast cancer.

Control selection

For selection of non-cases (for simplicity, "Controls") in the nested case-control design, cohort risk-set-based (incidence-density) sampling was conducted from the cohort pool of all eligible women at risk at the time of each case's diagnosis. Two control subjects were matched to each case on age (± 2 years) and year (same decade) at serum collection, and city (Hiroshima vs. Nagasaki). Controls were counter matched on radiation dose to improve efficiency for studying joint effects of radiation and the serum markers given the low frequency of high doses among controls (Cologne, Sharp, et al. 2004). Three dose strata (low, medium, and high) were defined based on tertile cutpoints of 5 mGy and 790 mGy among cases. Each sampled risk set contained the case as well as one control from each of the two dose strata not occupied by the case. Some sets had less than two controls due to unrecoverable samples or determining that a sample was drawn before menopause. This

sampling method does not preclude the possibility of a control being selected that could later become a case, however, in the period under surveillance, none of women selected as controls suffered a breast cancer. A total of 109 controls were selected (52 sets with two controls, 5 sets with only one control).

Determination of menopausal status

Menopausal status was determined using a conservative age range supplemented by an FSH (follicular stimulating hormone) assay. Samples collected at ages less than 43 were assumed to be from women who were premenopausal and not eligible; samples collected at age 55 and older were assumed postmenopausal (n=83 controls, n=44 cases). In the intermediate age range, serum FSH was measured and used to assign menopause status (n=27 controls, n=13 cases) based upon the manufacturer's test guidelines and the investigators' experience (postmenopausal cut-off: FSH \geq 23 mIU/ml) (SRL 2013).

Radiation Exposure

Radiation doses to the breast were based on the DS02 dosimetry system (Young & Kerr 2005), and were reported as weighted absorbed dose in gray (Gy) calculated as the gamma dose plus 10 times the neutron dose. Doses were adjusted to account for random errors as is routinely done within this cohort (Pierce et al. 1990).

Other data collection

Sera were stored in vials at -80°C . Samples were assayed for total estradiol, bioavailable estradiol (bE₂, total estradiol concentration multiplied by the percent of estradiol free or loosely bound to albumin), testosterone, progesterone, prolactin, ferritin, and (if in the designated age range) FSH. All assays were performed blinded to outcome and radiation exposure. Commercial laboratories with internal quality assurance systems performed all assays. Self-reported data on menopause and reproductive factors were available from a series of in-clinic questionnaires and mailed questionnaires. Body mass index (BMI, kg/m²) was measured at the time of serum collection. A full description of these methods is presented elsewhere (Grant et al. 2011) and a timeline of data collection is shown in Figure 2.

Methods of analysis

We used excess relative risk (ERR) models to estimate radiation risk (Thompson et al. 1994; Preston et al. 2007) with correction for the counter-matched sampling using a weighted analysis (Langholz & Borgan 1995). We used a binary odds model

$$\frac{p}{1-p} = e^{\eta_{j(i)} + \delta Z_{j(i)} r(bE_{2,j(i)}, d_{j(i)})}$$

where p is the probability of having breast cancer, $\mathbf{z}_{j(i)}$ is a vector of other risk factors (including other hormones) or confounders for individual j in risk set i with corresponding vector of parameters δ , $\eta_{j(i)}$ is the sampling weight (inverse of sampling probability from counter matching), and $d_{j(i)}$ is radiation dose. Risks for bE₂ and other factors (apart from

radiation dose) were estimated as natural log relative risks. Because of the risk set matching, model (1) is fit using conditional binary regression. Our primary risk model used to assess main effects of radiation and hormones, as well as interactions, was one with an excess relative risk for radiation and a log relative risk for natural-log-transformed bE₂ level (dropping the subscript $j(i)$ to simplify notation):

$$r(bE_2, d) = e^{\alpha \ln\{bE_2\}} \left(1 + \beta d e^{\gamma \ln\{bE_2\}} \right)$$

where β is the excess relative risk per Gy radiation dose, α is the log of relative risk for a one-unit difference in natural-log-transformed bE₂ level, and γ quantifies multiplicative statistical interaction between radiation and bE₂ level. Lack of interaction corresponds to $\gamma=0$, but lack of mechanistic interaction is sometimes equated with additivity of effects. We therefore used a general (mixture) model to assess the relative goodness of fits of multiplicative and additive parameterizations for the joint effect of bE₂ and radiation:

$$r(bE_2, d) = e^{\alpha \ln\eta} \left[\left(e^{\alpha \ln\{bE_2\}} (1 + \beta d) \right)^\theta \left[1 + e^{\alpha \ln\{bE_2\}} + \beta d \right]^{1-\theta} \right]$$

The left bracketed term represents a multiplicative parameterization while the right term is additive. Model (3) is similar to that used by Land et al (Land, Hayakawa, Machado, Y. Yamada, Pike, Akiba & Tokunaga 1994b). The conditional binary regression module “PECAN” of the Epicure software was used to fit the binary odds risk models (2) and (3) (Preston et al. 2015).

Causal modeling methods to assess mediation (Pearl 2009; Lange & Hansen 2011) cannot be immediately applied to counter-matched, nested case-control data. Therefore, to consider possible mediation of the effects of radiation through a bE₂ pathway (Fig. 1), we used a classical path analysis comparing risks for radiation with and without concomitant adjustment for the potential mediator in the binary regression model (Hafeman & Schwartz 2009; Huang et al. 2004). The estimate of mediation proportion is the difference between non-adjusted and adjusted values of the ERR for radiation, divided by the non-adjusted ERR. This is a valid estimate of causal mediation proportion under an assumption of no interaction between exposure and mediator (VanderWeele & Vansteelandt 2010). We tested mediation nonparametrically by randomly permuting hormone values among the matched sets, maintaining case-control identity of the hormone values to preserve the main effect of estradiol (5000 permutations) (Good 2006). This permutation simulates the distribution of mediation proportion under the null hypothesis of no mediation because permuting the hormone values but not the doses negates the association between radiation and hormone level. We also conducted the permutation test with simultaneous adjustment for the other hormone values, in which case the entire set of hormone values as a unit was permuted among matched sets. Permutation tests of mediation were performed in R using the conditional binary regression function “clogit” for a logistic approximation to model (1) and

the generalized nonlinear modeling function “gnm” for fitting model (2) (R Core Team 2014).

Biosamples were collected with oral informed consent in an era prior to that requiring explicit written informed consent. All current biosamples collection at RERF is performed using written informed consent. The RERF Human Investigation Committee approved this study. Privacy and confidentiality of subjects was regarded according to the Declaration of Helsinki.

Results

Characteristics of the study population are described in Table 1. The average age at diagnosis of postmenopausal breast cancer was 70.7 years. Serum samples were collected an average of 11.6 years prior to diagnosis, and 11.7 years prior to the matched index case for controls.

We examined the correlations between the log-transformed hormone levels. Only testosterone and progesterone had moderate correlations with bE_2 (0.32 and 0.50, respectively). The only other correlations of note were IGF-1 and IGFBP-3 with a correlation value of 0.52 and progesterone and testosterone with a correlation of 0.25. All other correlations were low (absolute value \leq 0.15).

Table 2 shows the distributions and univariate (crude, not adjusted for other factors) relative risks (RR) estimated using model (2) for breast cancer risk factors considered in this study. Significantly reduced risks were found for women who were older at menarche (RR=0.57 per year older; 95% CI: 0.34, 0.83, $p=0.003$) and who had more full term pregnancies (RR=0.71 per full term pregnancy; 95% CI: 0.55, 0.90; $p=0.003$), while a higher risk was observed per unit increase in BMI (1.12; 95% CI: 1.01, 1.25; $p=0.035$). Although other associated risks were not statistically significant, cases were more likely to be nulliparous (13% vs 8%), to be older than controls at menopause (48.8 vs 47.1 years), and older at time of first birth (23.3 vs 22.9 years).

Mean radiation dose to the breast in cases was 0.50 Gy while mean dose in non-cases was 0.33 Gy (corrected for stratified counter-matching as explained in Footnote ‘e’ of Table 2). Radiation dose was significantly associated with breast cancer risk (ERR = 0.61/Gy; 95% CI: 0.00, 1.83; $p=0.049$ via model (2) with no interaction [$\gamma=0$] and no adjustment for other factors). Radiation risk declined slightly with increasing age at exposure and increasing attained age, as in previous analyses of the atomic bomb survivor cohort (Tokunaga et al. 1994; Preston et al. 2007), but neither effect modifier was statistically significant in this study (data not shown).

We assessed the breast cancer risk of individual hormone levels using model (2) with no adjustment for radiation dose. A significant increase in RR per unit increase in log bE_2 was observed (RR=2.04; 95% CI: 1.04, 4.09; $p=0.029$). Unit increases in levels of both log testosterone and log progesterone showed positive, significant associations with breast cancer risk (RR=1.85 95% CI: 1.06, 3.31; $p=0.030$ and RR=6.31 95% CI: 2.27, 19.1;

$p < 0.001$, respectively). No significant associations with levels of log prolactin, IGF-1, IGFBP-3, or log ferritin were observed.

The relative change with radiation exposure of log bE_2 levels at 1 Gy was 1.20 (95% CI: 0.98, 1.41; $p = 0.072$) among cases and 1.19 (95% CI: 1.05, 1.34; $p = 0.010$) among controls. The mean log bE_2 was 2.33 pg/ml among cases (i.e. the geometric mean bE_2 level was $e^{2.33} = 10.3$ pg/ml), while the mean log among controls was 2.12 pg/ml (adjusted for the radiation-related sampling due to the association between radiation and bE_2 levels among cancer-free women).

We analyzed the joint effects of radiation and bE_2 using the mixture model (3) to investigate if an additive or multiplicative model best fit the data. Setting the mixture parameter (θ) to 0 produces estimates for a pure additive model (deviance 123.344) while a pure multiplicative model ($\theta = 1$) fit slightly better (deviance 122.409). An interaction model (2) was used to test whether bE_2 significantly modified the radiation response (i.e. $\gamma \neq 0$). The estimate of the interaction term was large and positive (3.86; 95% CI: 0.27, >2000; $p = 0.32$), suggesting a super-multiplicative joint effect, but was not significant (deviance 121.403). Main and joint relative risks estimated from these three models are shown using a 2×2 table layout in Figure 3.

Based on model (2) without interaction, the mediation (proportion of total radiation risk explained by bE_2) on the log RR scale ($\log RR = \log[1 + ERR]$) was 14% (ERR was 0.467 with adjustment for log bE_2 , 0.605 without). The permutation test p -value was 0.004. Analogous tests of mediation of the log RR for radiation by testosterone, progesterone, and prolactin (without adjustment for bE_2 or other hormones) conducted post-hoc revealed 17% ($p = 0.005$), 11% ($p = 0.12$), and 0.8% ($p = 0.21$) decreases, respectively. With further adjustment for these other hormones there was no evidence of mediation by bE_2 (0.2% decrease in log RR for radiation; $p = 0.47$).

Discussion

Ionizing radiation and high levels of bE_2 are known breast cancer (BC) risk factors. The atomic bomb survivor cohort provides a rare opportunity to study these factors simultaneously in humans. We observed that 1 Gy of ionizing radiation exposure to the breast raised the risk of postmenopausal BC risk by about 60%. A higher level of postmenopausal bE_2 was also associated with increased risk of postmenopausal BC. The effects of traditional BC risk factors conformed to expectations, although—as might be expected in a study of this size—not all were statistically significant. The joint effects of IR and bE_2 were statistically consistent with both additive and multiplicative models; it is known that power to discriminate the scale of joint effects can be low (Cologne, Pawel, et al. 2004; Greenland 1983). While not statistically significant, the point estimate of the interaction term and the results from the mixture model both indicated that a multiplicative model provided a slightly better fit.

In a previous study of female cancer-free atomic bomb survivors, we found that radiation exposure was associated with higher levels of bE_2 in 199 postmenopausal samples (Grant et

al. 2011). In the present study, in which the controls are a subset of the previous study, we observed a similar effect of radiation on bE₂ level among both cases and controls, although they were not statistically significant. Using a classical path analysis we observed a modest decrease (14%) in the risk of radiation exposure after adjusting for postmenopausal bE₂ level, indicating that bE₂ level may have a mediating effect upon the radiation risk of postmenopausal breast cancer. Although a classical path analysis is not ideal for assessing mediation with fundamentally nonlinear regression models such as those used for cohort or case-control analyses (Lange et al. 2012), the counter-matched nested case-control design employed in the present study prohibits immediate application of more appropriate methods of mediation analysis. This is a topic of ongoing methodological work.

There is a strong consensus that the direct pathway for increased risk of solid cancer (including breast cancer) after radiation exposure is DNA damage, particularly double strand breaks, which are often repaired with error-prone mechanisms that lead to deletion mutations and genomic instability. There is also a body of knowledge showing that systems within the body can be altered by radiation exposure (i.e., indirect effects). Examples include changes in immune response resulting in a decline of T-cell immunity leading to increased inflammation (Hayashi et al. 2005), altered lipid metabolism (Wong et al. 1999; Bezemer et al. 2005; Akahoshi et al. 2003), and increased fatty liver and hypertension, among others (M. Yamada et al. 2004). There is evidence that indirect effects contribute to increased risks of non-cancer diseases and circulatory-related mortality (Shimizu et al. 2010; Ozasa et al. 2012). Mediation, such as that by estradiol suggested in the present study, may therefore be an aspect of radiation carcinogenesis requiring further investigation.

The results of the present study are generally consistent with the findings of Land et al, (Land, Hayakawa, Machado, Y. Yamada, Pike, Akiba & Tokunaga 1994b) who noted that radiation exposure interacted with reproductive factors that act to reduce overall exposure to high levels of endogenous sex hormones and/or reduce the number of undifferentiated mammary gland cells that are sensitive to sex hormones and radiation damage. Brooks et al. described higher risk of contralateral breast cancer if the woman was nulliparous at the time of radiation treatment for the initial breast cancer while women who were parous showed no additional risks (Brooks et al. 2012). Pregnancy is thought to protect the breast against cancer due to the advancement of undifferentiated breast lobules to more developed ones (J. Russo et al. 2005). Studies exploring the effects of reproductive status at the time of exposure have reported varying findings. Holmberg reported no interaction of radiation risks and fertility patterns for breast cancer risk among women who had been irradiated for skin hemangiomas in infancy (Holmberg 2001). Ronckers et al. failed to see a difference in BC risk among women with scoliosis exposed to diagnostic X-rays at different reproductive development periods (Ronckers et al. 2008) while McDougall et al. did not see a hypothesized increase in BC risk among atomic bomb survivors exposed between menarche and first birth compared to other developmental periods (McDougall et al. 2010). Hill et al. found higher risks of BC among women treated during pregnancy for Hodgkin lymphoma (Hill et al. 2005).

An early rat study indicated synergistic effects of estradiol and IR (Segaloff & Maxfield 1971). In another study, rats that underwent ovariectomy showed no radiation risks

compared to normal rats, while those treated with estradiol showed higher radiation risks (Solleveld et al. 1986). Pedram et al (Pedram et al. 2009) suggested a possible mechanism whereby estradiol interferes with the signalling and cell cycle checkpoint processes that occur after DNA damage, which may partially explain a synergistic interaction between bE_2 and radiation exposure.

There are several limitations to this study. The number of cancer cases is small, levels of mediation were modest, and power to assess effect modification was limited. Menopausal status at time of sample collection was based on age and FSH level. FSH has been shown to be not fully specific (Henrich et al. 2006) so some misclassification of menopause status among the 25% of samples that were assigned using FSH is possible. Mediation analysis for dichotomous outcome data can produce misleading results if there is interaction between the exposure and mediator. Generalizability of the findings may be limited due to the fact that the subjects received whole-body exposure, which is different than exposures to the breast during mammograms for breast cancer screening. Another generalizability issue is the fact that these women survived at least 25 years after an acute whole-body exposure prior to having sera collected. To perform the permutation tests, we used either conditional logistic regression or unconditional nonlinear binary odds regression, but all approaches gave similar results.

The study has several important strengths. Most notably, it was prospective with biosamples collected uniformly for all members of the cohort regardless of outcome or exposure, and prior to diagnosis for cases. Dosimetry is exceptionally reliable for a population-based study. Inferences concerning hormonal involvement were based on sera measurements rather than self-reported reproductive factors. Permutation tests are non-parametric and do not rely on any particular assumed distribution for the test statistic (Good 2006).

Conclusions

This study of women exposed to atomic bomb radiation shows increased risk of postmenopausal breast cancer due to radiation as well as to circulating levels of bE_2 . A portion of the risk of radiation exposure appeared to be mediated by bE_2 levels. Estimates of the joint effects of radiation and bE_2 provided only equivocal evidence regarding effect modification by bioavailable estradiol levels. This is the first direct epidemiologic evidence of hormonal effects on the magnitude of radiation-induced breast cancer risk; the finding requires replication and possibly further elucidation by genomic and metabolomic studies in radiation-exposed cohorts.

Acknowledgements:

We thank the women of the AHS study for graciously donating biosamples. We also thank Dr. Roy Shore for his many helpful comments and suggestions and Dr. Kazuo Neriishi for his assistance and advice. The Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan is a public interest foundation funded by the Japanese Ministry of Health, Labour and Welfare and the US Department of Energy (DOE). The research was also funded in part through DOE award DE-HS0000031 to the National Academy of Sciences, and by US National Cancer Institute contracts HHSN261200900005C and HHSN261201400009C, and the Japanese Ministry of Education, Culture, Sports, Science and Technology grants 14031227 and 15026220, and 25330039 (S Izumi). The views of the authors do not necessarily reflect those of the two funding governments.

References

- Akahoshi M et al., 2003 Effects of radiation on fatty liver and metabolic coronary risk factors among atomic bomb survivors in Nagasaki. *Hypertension research: official journal of the Japanese Society of Hypertension*, 26(12), pp.965–970. [PubMed: 14717339]
- Bauer S et al., 2005 Radiation exposure due to local fallout from Soviet atmospheric nuclear weapons testing in Kazakhstan: solid cancer mortality in the Semipalatinsk historical cohort, 1960–1999. *Radiation Research*, 164(4 Pt 1), pp.409–419. [PubMed: 16187743]
- Bezemer ID et al., 2005 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*, 16(5), pp.561–572. [PubMed: 15986111]
- Boice JD et al., 1991 Frequent chest X-ray fluoroscopy and breast cancer incidence among tuberculosis patients in Massachusetts. *Radiation Research*, 125(2), pp.214–222. [PubMed: 1996380]
- Brooks JD et al., 2012 Reproductive status at first diagnosis influences risk of radiation-induced second primary contralateral breast cancer in the WECARE study. *International journal of radiation oncology, biology, physics*, 84(4), pp.917–924.
- Carmichael A, Sami AS & Dixon JM, 2003 Breast cancer risk among the survivors of atomic bomb and patients exposed to therapeutic ionising radiation. *European journal of surgical oncology: the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology*, 29(5), pp.475–479.
- Cologne JB, Pawel DJ, et al., 2004 Uncertainty in estimating probability of causation in a cross-sectional study: joint effects of radiation and hepatitis-C virus on chronic liver disease. *Journal of radiological protection*, 24(2), pp.131–145. [PubMed: 15296257]
- Cologne JB, Sharp GB, et al., 2004 Improving the efficiency of nested case-control studies of interaction by selecting controls using counter matching on exposure. *International Journal of Epidemiology*, 33(3), pp.485–492. [PubMed: 15105408]
- Good PI, 2006 *Resampling Methods* 3rd ed, Boston: Birkhäuser.
- Grant EJ et al., 2011 Associations of ionizing radiation and breast cancer-related serum hormone and growth factor levels in cancer-free female A-bomb survivors. *Radiation Research*, 176(5), pp.678–687. [PubMed: 21718103]
- Greenland S, 1983 Tests for interaction in epidemiologic studies: a review and a study of power. *Statistics in medicine*, 2(2), pp.243–251. [PubMed: 6359318]
- Hafeman DM & Schwartz S, 2009 Opening the Black Box: a motivation for the assessment of mediation. *International Journal of Epidemiology*, 38(3), pp.838–845. [PubMed: 19261660]
- Hayashi T et al., 2005 Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. *The American journal of medicine*, 118(1), pp.83–86. [PubMed: 15639214]
- Henrich JB et al., 2006. Limitations of follicle-stimulating hormone in assessing menopause status: findings from the National Health and Nutrition Examination Survey (NHANES 1999–2000). *Menopause (New York, NY)*, 13(2), pp.171–177.
- Hill DA et al., 2005 Breast cancer risk following radiotherapy for Hodgkin lymphoma: modification by other risk factors. *Blood*, 106(10), pp.3358–3365. [PubMed: 16051739]
- Holmberg E, 2001 Excess breast cancer risk and the role of parity, age at first childbirth and exposure to radiation in infancy. *British journal of cancer*, 85(3), pp.362–366. [PubMed: 11487266]
- Howe GR & McLaughlin J, 1996 Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study and a comparison with breast cancer mortality in the atomic bomb survivors study. *Radiation Research*, 145(6), pp.694–707. [PubMed: 8643829]
- Huang B et al., 2004 Statistical assessment of mediational effects for logistic mediational models. *Statistics in medicine*, 23(17), pp.2713–2728. [PubMed: 15316954]
- Kabuto M et al., 2000 A prospective study of estradiol and breast cancer in Japanese women. *Cancer epidemiology, biomarkers & prevention*, 9(6), pp.575–579.
- Land CE, Hayakawa N, Machado SG, Yamada Y, Pike MC, Akiba S & Tokunaga M, 1994a A case-control interview study of breast cancer among Japanese A-bomb survivors. I. Main effects.

- Cancer Causes & Control, 5(2), pp.157–165. Available at: http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Retrieve&list_uids=8167263&dopt=abstractplus. [PubMed: 8167263]
- Land CE, Hayakawa N, Machado SG, Yamada Y, Pike MC, Akiba S & Tokunaga M, 1994b A case-control interview study of breast cancer among Japanese A-bomb survivors. II. Interactions with radiation dose. *Cancer Causes & Control*, 5(2), pp.167–176. [PubMed: 8167264]
- Lange T & Hansen JV, 2011 Direct and indirect effects in a survival context. *Epidemiology* (Cambridge, Mass), 22(4), pp.575–581.
- Lange T, Vansteelandt S & Bekaert M, 2012 A simple unified approach for estimating natural direct and indirect effects. *American Journal of Epidemiology*, 176(3), pp.190–195. [PubMed: 22781427]
- Langholz B, 2005 Case-Control Study, Nested. In *Encyclopedia of Biostatistics*. pp. 646–655.
- Langholz B & Borgan OR, 1995 Counter-matching: A stratified nested case-control sampling method. *Biometrika*, 82(1), pp.69–79.
- Mabuchi K et al., 1994 Cancer incidence in atomic bomb survivors. Part I: Use of the tumor registries in Hiroshima and Nagasaki for incidence studies. *Radiation Research*, 137(2 Suppl), pp.S1–16. [PubMed: 8127951]
- McDougall JA et al., 2010 Timing of menarche and first birth in relation to risk of breast cancer in A-bomb survivors. *Cancer Epidemiology Biomarkers & Prevention*, 19(7), pp.1746–1754.
- Ostroumova E et al., 2008 Breast cancer incidence following low-dose rate environmental exposure: Techa River Cohort, 1956–2004. *British journal of cancer*, 99(11), pp.1940–1945. [PubMed: 19002173]
- Ozasa K et al., 2012 Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: an overview of cancer and noncancer diseases. *Radiation Research*, 177(3), pp.229–243. [PubMed: 22171960]
- Pearl J, 2009 Causal inference in statistics: An overview. *Statistics Surveys*, 3, pp.96–146.
- Pedram A et al., 2009 Estrogen inhibits ATR signaling to cell cycle checkpoints and DNA repair. *Molecular biology of the cell*, 20(14), pp.3374–3389. [PubMed: 19477925]
- Pierce DA, Stram DO & Vaeth M, 1990 Allowing for random errors in radiation dose estimates for the atomic bomb survivor data. *Radiation Research*, 123(3), pp.275–284. [PubMed: 2217725]
- Preston DL et al., 2015 *Epicure User Guide* 12 ed, Ottawa: Risk Sciences International.
- Preston DL et al., 2010 How much can we say about site-specific cancer radiation risks? *Radiation Research*, 174(6), pp.816–824. [PubMed: 21128806]
- Preston DL et al., 2007 Solid Cancer Incidence in Atomic Bomb Survivors: 1958–1998. *Radiation Research*, 168(1), pp.1–64. [PubMed: 17722996]
- R Core Team, 2014 *R: A Language and Environment for Statistical Computing* 2nd ed., Vienna, Austria Available at: <http://www.R-project.org>.
- Ronckers CM et al., 2008 Multiple diagnostic X-rays for spine deformities and risk of breast cancer. *Cancer epidemiology, biomarkers & prevention*, 17(3), pp.605–613.
- Russo IH & Russo J, 2011 Pregnancy-induced changes in breast cancer risk. *Journal of mammary gland biology and neoplasia*, 16(3), pp.221–233. [PubMed: 21805333]
- Russo J et al., 2005 The protective role of pregnancy in breast cancer. *Breast Cancer Research*, 7(3), pp.131–142. [PubMed: 15987443]
- Segaloff A & Maxfield WS, 1971 The synergism between radiation and estrogen in the production of mammary cancer in the rat. *Cancer research*, 31(2), pp.166–168. [PubMed: 5545267]
- Shimizu Y et al., 2010 Radiation exposure and circulatory disease risk: Hiroshima and Nagasaki atomic bomb survivor data, 1950–2003. *British Medical Journal*, 340(jan14 1), pp.b5349–b5349. [PubMed: 20075151]
- Sigurdson AJ et al., 2003 Cancer incidence in the US radiologic technologists health study, 1983–1998. *Cancer*, 97(12), pp.3080–3089. [PubMed: 12784345]
- Solleveld HA et al., 1986 Effects of X-irradiation, ovariectomy and estradiol-17 beta on incidence, benign/malignant ratio and multiplicity of rat mammary neoplasms—a preliminary report. *Leukemia research*, 10(7), pp.755–759. [PubMed: 3736110]

- SRL (Tokyo Special Reference Laboratories, Inc) 2013, 卵胞刺激ホルモン (FSH) 基準値 [Follicle-stimulating hormone (FSH) reference levels]. Available at: http://www.srl.info/srlinfo/kensa_ref_CD/otherdata/06-6118.html [Accessed October 24, 2013].
- Thompson DE et al., 1994 Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. *Radiation Research*, 137(2 Suppl), pp.S17–67. [PubMed: 8127952]
- Tokunaga M et al., 1994 Incidence of female breast cancer among atomic bomb survivors, 1950–1985. *Radiation Research*, 138(2), pp.209–223. [PubMed: 8183991]
- VanderWeele TJ & Vansteelandt S, 2010 Odds ratios for mediation analysis for a dichotomous outcome. *American Journal of Epidemiology*, 172(12), pp.1339–1348. [PubMed: 21036955]
- Wong FL et al., 1999 Effects of radiation on the longitudinal trends of total serum cholesterol levels in the atomic bomb survivors. *Radiation Research*, 151(6), pp.736–746. [PubMed: 10360794]
- Yamada M et al., 2004 Noncancer disease incidence in atomic bomb survivors, 1958–1998. *Radiation Research*, 161(6), pp.622–632. [PubMed: 15161358]
- Young RW & Kerr GD eds., 2005 Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki - Dosimetry System 2002, Hiroshima: Radiation Effects Research Foundation.

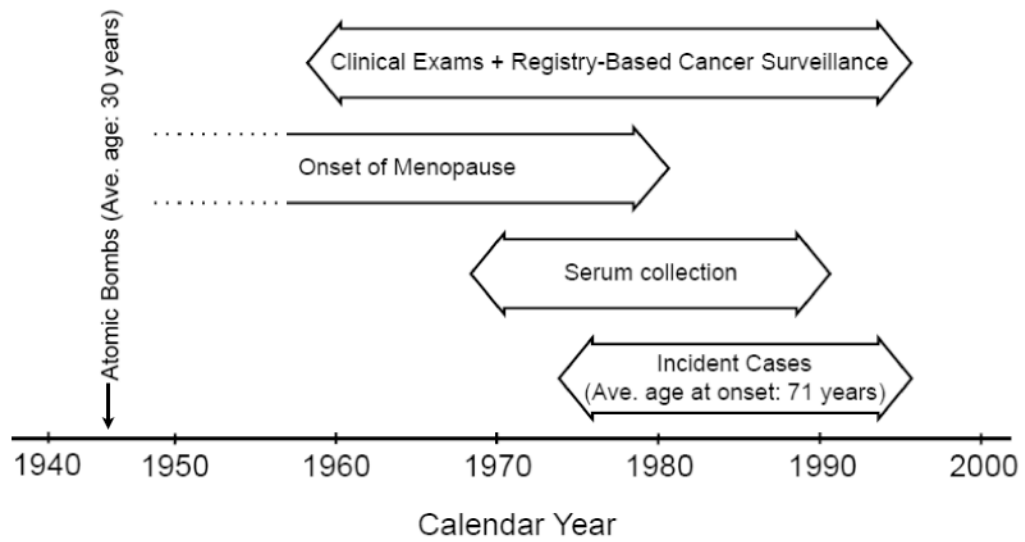


Figure 1.

Timeline of study. On average, subjects for this study were 30 years of age at the time of the bombings (1945) and 71 years of age at the time of breast cancer diagnosis. Cancer surveillance and clinical exams began in 1958. Serum collection began in 1969 and all samples were collected at least two years prior to diagnosis. Cases in this study were diagnosed between 1974 and 1996.

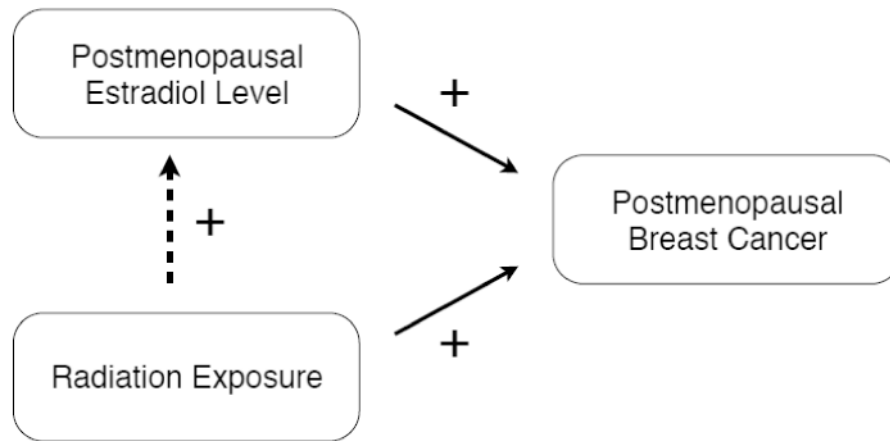


Figure 2. Both Radiation exposure and postmenopausal level of estradiol are known breast cancer carcinogens. Previous findings indicated that radiation exposure can increase postmenopausal levels of estradiol in cancer-free women (dotted line). The research question is therefore whether estradiol mediates the radiation risk of postmenopausal breast cancer.

		Bioavailable Estradiol	
		-	+
Radiation	-	1.0 (reference)	1.86 (0.87, 3.98; p=0.11) Additive 1.83 (0.98, 3.62; p=0.06) Multiplicative 1.43 (0.55, 3.28) Interaction
	+	1.41 (0.87, 2.55; p=0.19) Additive 1.47 (0.96, 2.57; p=0.10) Multiplicative 1.30 (1.00, 2.40) Interaction	2.27 ^a (deviance: 123.344) Additive 2.69 ^b (deviance: 122.409) Multiplicative 7.18 ^c (deviance: 121.403) Interaction

^a $1 + 0.41 + 0.86$
^b $1.47 * 1.83$
^c $1.30 * 1.43 * 3.86$ (interaction term)

Figure 3.

Two-by-two representation of the joint effects of bioavailable estradiol and radiation exposure based on additive, multiplicative and interaction models. Estradiol levels in the reference group (“-”) were the geometric mean among the control subjects and the level in the “exposed” group (“+”) was one log unit above the geometric mean (i.e. 2.72-times higher on a linear scale). Radiation dose in the unexposed group was 0 Gy, and it was 1 Gy for the “exposed” group. The main effects were estimated using additive, multiplicative, and interaction models (without adjustment for other variables), and then the respective joint effects were manually calculated (see footnotes) and displayed in the bottom-right quadrant. The smallest deviance was observed using the interaction model.

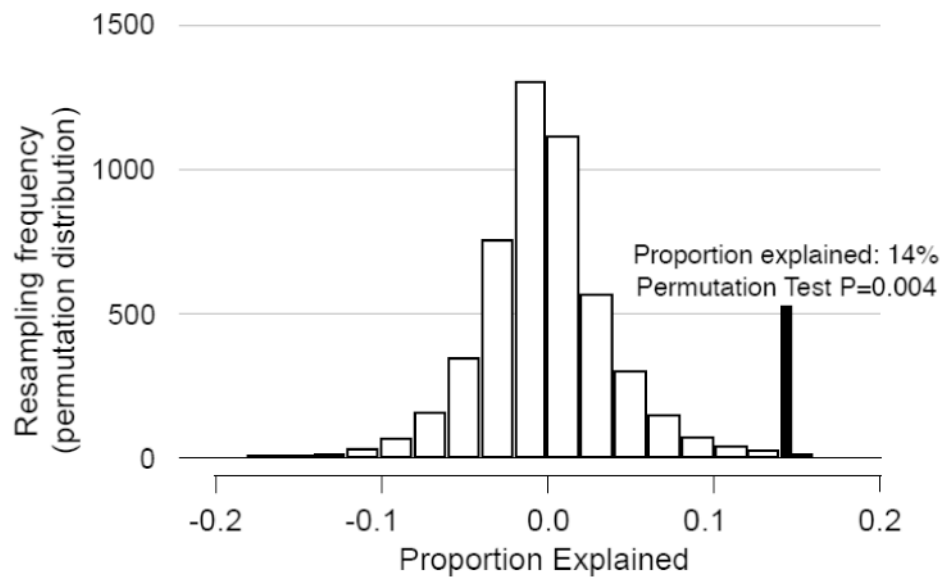


Figure 4.

Results of a permutation test to determine if the effects of radiation were mediated through changes in the level of bioavailable estradiol (bE2). Hormone values were randomly permuted (5000 permutations) among case-control sets maintaining case status of the values; this preserves the main effects of bE₂ levels and radiation while rendering bE₂ levels random with respect to radiation dose (which would be true under the null hypothesis of no mediation). The distribution of the proportion explained ($[\log(\text{radiation risk}) - \log(\text{radiation risk after adjustment of bE}_2)] / \log(\text{radiation risk})$). The solid line shows the actual proportion explained in the dataset (14%), which was outside of the expected range at a p-level of 0.004 (via permutation testing). This indicates that there was statistically significant evidence of mediation of the radiation risk via bE2 level changes.

Table 1.

Subject characteristics.

Characteristic (mean, SD, 5th percentile, 95th percentile)	Cases	Controls
Number of subjects	57	109
	70.7 (9.6)	70.4 ^a (9.2)
Age at diagnosis (years)	56.9, 89.5	56.0, 88.0
	30.2 (8.6)	29.8 (8.2)
Age at radiation exposure (years)	14.0, 44.0	16.0, 43.0
	59.2 (7.7)	58.9 (7.6)
Age at sample collection (years)	45.6, 72.7	45.9, 70.9
Time from sample collection to case diagnosis (years)	11.6 (6.8)	11.7 ^a (6.7)
	3.0, 24.0	3.3, 24.5

^aValues for controls calculated using their age at the time of the index case's diagnosis

Table 2.

Distributions and univariate relative risks of considered breast cancer risk factors.

Factor	Mean value (SD) 5th percentile, 95th percentile Number with known data		Relative risk [95% CI] p-value	
	Cases N=57	Non-cases N=109		
Age at menarche (years)	15.1 (1.7)	15.5 (1.8)		0.57^a
	12, 18	13, 18		[0.34, 0.83]
	32	79		p=0.003
Age at menopause (years)	48.8 (4.0)	47.1 (5.0)		1.08 ^a
	42, 53	40, 54		[0.96, 1.23]
	35	81		p=0.22
Nulliparous (proportion)	0.13 (0.34)	0.08 (0.27)		2.01 ^b
	–	–		[0.48, 9.12]
	53	105		p=0.34
Number of full-term pregnancies	2.6 (1.9)	3.4 (1.9)		0.71^a
	0, 6	0, 7		[0.55, 0.90]
	53	105		p=0.003
Number of full-term pregnancies	Frequency (prop.)	Frequency		Heterogeneity P = 0.007
0		(prop.)		
	7 (0.13)		1.00	(reference)
1		8 (0.08)		
	13 (0.25)		1.69	[0.31, 10.5] p>0.5
2		7 (0.07)		
	8 (0.15)		0.43	[0.07, 2.44] p=0.34
3		19 (0.18)		
	9 (0.17)		0.17	[0.02, 1.16] p=0.071
4+ (mean 5.2)	16 (0.30)	25 (0.24)	0.18	[0.03, 0.95] p=0.043
		46 (0.44)		
Age at first full-term pregnancy	23.3 (3.3)	22.9 (3.5)		1.07 ^a
(years)	20, 30	19, 30		[0.94, 1.24]
	40	89		p=0.30
Body mass index (kg/m ²)	23.3 (3.7)	22.6 (3.3)		1.12^{a,d}
	17.0, 28.5	17.1, 28.3		[1.01, 1.25]
	54	103		p=0.035
Body mass index categories (kg/m ²)	Frequency (prop.)	Frequency (prop.)		Heterogeneity p=0.21
< 20	11 (0.20)	22 (0.21)	1.70	[0.53, 5.49] p=0.37
20, <22.5	12 (0.22)	31 (0.30)	1.00	(reference)
22.5, <25	12 (0.22)	24 (0.23)	1.72	[0.49, 5.99] p=0.39

Factor	Mean value (SD) 5th percentile, 95th percentile Number with known data		Relative risk [95% CI] p-value		
	Cases N=57	Non-cases N=109			
25, <27.5	11 (0.20)	18 (0.17)	2.79	[0.81, 9.98]	p=0.10 ^c
27.5	8 (0.15)	8 (0.08)	4.72	[1.22, 19.4]	p=0.025
Biomarkers (serum)					
Log bioavailable E2 (pg/ml)	2.33 (0.73)	2.12 (0.64) ^e	2.04	[1.07, 4.09]	p=0.029
Log testosterone (ng/dl)	2.59 (0.50)	2.38 (0.67) ^e	1.85	[1.06, 3.31]	p=0.030
Log progesterone (ng/ml)	-1.12 (0.47)	-1.34 (0.39) ^e	6.31	[2.27, 19.1]	P<0.000
Log prolactin (ng/ml)	1.28 (0.57)	1.21 (0.59) ^e	1.08	[0.60, 1.96]	p>0.5
IGF-1 (ng/ml)	101.9 (45.8)	112.1 (35.2) ^e	1.00	[0.99, 1.01]	p>0.5
IGFBP-3 (µg/ml)	2.39 (0.47)	2.45 (0.40) ^e	0.86	[0.38, 2.07]	p>0.5
Log ferritin (ng/ml)	4.06 (0.88)	3.93 (0.73) ^e	1.34	[0.88, 2.08]	p=0.18
Radiation dose (1 Gy)	0.50 (0.89)	0.33 (0.54) ^e	1.61^f	[1.00, 2.83]	P=0.049

^aWith continuous variables, the relative risk is multiplicative per increasing unit change. (For example, the RR is reduced by a factor of 0.57 for each year menarche is delayed. Therefore a women having menarche two years later than another women would have 32% [$0.57^2 \text{ years} = 0.32$] the risk.)

^bReference is one or more full-term deliveries [nulliparous estimate]

^cRelative risk for "overweight" (BMI> 25) is 3.43 (95% CI: 1.14, 11.1, p= 0.029).

^dA quadratic term in BMI was not significant (P>0.5).

^eDue to counter matching (stratified sampling of controls within risk sets), a weighted mean for non-case samples was computed using the risk-set-specific and stratum-specific sampling weights. Although only bioavailable E2 and testosterone have demonstrated relationships to radiation dose, due to some correlation between hormones, the weighted values are shown for all hormones in non-cases.

^fCalculated as ERR using Equation 1 ($RR = ERR + 1$). The ERR is additive per increasing unit change in dose. (For example, the ERR increases by an increment of 0.50 per Gy. Therefore a woman exposed to 2 Gy would have an ERR of 2.0 or a RR of 3.0.)