

REPORTS

Evaluation of the Digital Rectal Examination as a Screening Test for Prostate Cancer

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Background: The utility of digital rectal examination (DRE) as a screening test for early detection of prostate cancer has not been established. Therefore, we evaluated the usefulness of DRE as a stand-alone screening test and in conjunction with measured serum prostate-specific antigen (PSA) levels of 0–3.9 ng/mL and transrectal ultrasonography (TRUS). **Methods:** Our study population consisted of 10 523 men aged 54–76 years who were randomly assigned to the screening arm of the Rotterdam, The Netherlands, section of the European Randomized Study of Screening for Prostate Cancer. The underlying prevalence of detectable prostate cancer was estimated by logistic regression analysis and used for calculating the sensitivity of DRE as a test. Pathologic characteristics of 105 radical prostatectomy specimens were used to determine the aggressiveness of the tumors diagnosed (and missed) by DRE. **Results:** The overall detection rate for prostate cancer in this population when serum PSA measurement, DRE, and TRUS were used was 4.5%, and the detection rate with DRE alone was 2.5%. The positive predictive value of DRE ranged from 4% to 11% in men with PSA levels of 0–2.9 ng/mL and from 33% to 83% in men with PSA levels of 3.0–9.9 ng/mL or more. Most tumors detected by DRE in men with

PSA levels of less than 4.0 ng/mL were small (mean volumes = 0.24–0.83 mL), and most were well differentiated (Gleason scores of 6 or less). Minimal, moderate, and advanced cancers were seen in 42%, 42%, and 16% of men, respectively, with a PSA level of 4.0 ng/mL or less. DRE alone allowed detection of 264 (55.8%) of 473 cancers; 82 (17.3%) of the 473 cancers would have remained undetected by PSA-based screening alone (i.e., no follow-up procedures for PSA values of 0–3.9 ng/mL). **Conclusions:** For PSA values of 0–3.9 ng/mL, the positive predictive value and sensitivity of DRE, tumor volume, and tumor grade were strongly dependent on PSA level. DRE has a poor performance in low PSA ranges. [J Natl Cancer Inst 1998;90:1817–23]

Screening for prostate cancer is an accepted health-care policy in some countries, but screening is fiercely opposed in others (1–3). Reports from areas where screening is prevalent show that the routine of prostate-specific antigen (PSA)-based early detection leads to a drastic initial rise in prostate cancer incidence—because of all the subclinical cancers detected—followed by a decrease once saturation has been reached (4). The effect of PSA screening on mortality, if one occurs, will become visible much later. At present, the value of screening for prostate cancer is still uncertain with respect to mortality reduction and quality-of-life effects.

Large randomized trials provide a means to avoid important biases and provide sufficient statistical power to obtain reliable data. Such an effort is going on in a number of European countries through the European Randomized Study of Screening for Prostate Cancer (ERSPC), which has established close cooperation with the American Screening Project for Prostate, Lung, Colon, and Ovarian Cancer (5) and the Canadian randomized study of screening for prostate cancer (6).

This report is based on data from the

screening arm of the Rotterdam, The Netherlands, section of the ERSPC. It is aimed at evaluating digital rectal examination (DRE) as a screening test for prostate cancer.

The value of DRE as a diagnostic screening test can be judged in several ways: by considering DRE as a stand-alone test, by looking at its incremental value, and by considering its value in conjunction with PSA values of 0–3.9 ng/mL and transrectal ultrasonography (TRUS). This report uses all three approaches; its main purpose, however, is to determine the contribution of DRE as a single test relative to that of PSA levels and tumor characteristics.

MATERIALS AND METHODS

European Randomized Study of Screening for Prostate Cancer

Data were obtained from the prevalence screen in the Rotterdam section of ERSPC for a 33-month period starting on July 1, 1994. The screening algorithm used was applied in a prospective fashion as part of the original protocol. The goals and structure of ERSPC are described elsewhere (7,8). A summary of the screening procedures and the participants is given in Fig. 1. Men of ages 54–70 years (in Rotterdam, the range was 54–76 years [one patient aged 54 years and one aged 76 years were included by accident]) are randomly assigned to screening or no screening. The main end point of the study is prostate cancer mortality. When this report was being written (March 1998), more than 30 000 men in Rotterdam, more than 110 000 men in Europe, and more than 190 000 men internationally through the International Prostate Screening Trial Evaluation Group had been randomly assigned.

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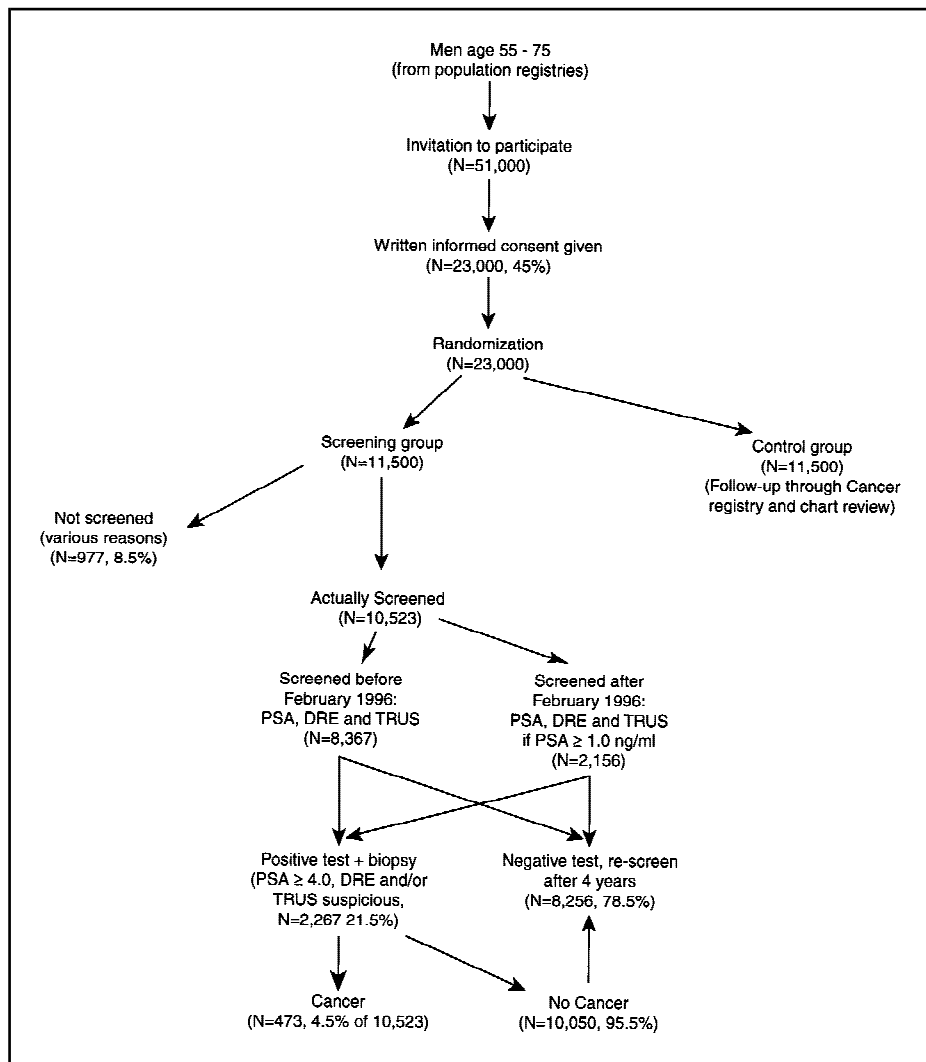


Fig. 1. CONSORT diagram—screening procedure and participants.

Study Population and Screening Procedures

Participants are recruited from the population registry of Rotterdam and from surrounding communities. The invitation to participate is by letter. Written informed consent is required by Dutch law. The participation rate (i.e., the proportion of those who have been invited to participate and have in fact been randomly assigned to screening) varies around 45%. The participation rate is defined as the number of men who have been randomly assigned to screening divided by the number of men who were invited to participate. Process evaluation was carried out. Questionnaires administered to participants and refusers alike indicated that the frequency of prostatic symptoms in the two groups was equal. This finding suggests no selection bias on this account. Rescreening is carried out once after 4 years in the ERSPC protocol.

This study is based on 11 500 participants in the Rotterdam section of ERSPC who were randomly assigned to the screening arm during the period from 1994 through 1997, of whom 10 523 (92%) were actually screened. In February 1996, the protocol was changed so that men who had PSA levels of less than 1.0 ng/mL were not screened by DRE and

TRUS. Of 3858 men with PSA values of less than 1.0 ng/mL, 1702 were recruited before this change and 2156 were recruited after this change (Fig. 1).

The screening algorithm called for a biopsy in all men who had at least one of the following results: an abnormal DRE, an abnormal TRUS, or a PSA level of 4.0 ng/mL or more. DRE findings were considered abnormal if nodularity, induration, or asymmetry was felt. TRUS findings were considered abnormal if hypoechoic lesions were seen. In March 1997, a major protocol change was implemented. All men with PSA levels of 3.0 ng/mL or more were subjected to a biopsy. DRE and TRUS were omitted if an individual's PSA level was less than 3.0 ng/mL. Data obtained within the new protocol are not included in this report.

DREs, TRUSs, and biopsies were carried out by trained medical and paramedical personnel. Training periods for these personnel lasted 4–6 weeks, and their normal and abnormal findings were randomly counterchecked by experienced staff. Trainees were permitted to do independent examination only after they and their instructors had achieved an acceptable level of agreement on diagnosis. Still, for eight examiners involved, percentages of abnormal DRE findings and positive predictive values varied between 4% and 24% and between 13% and 34%,

respectively. Prostatic biopsies were taken as sextant biopsies. The lateral biopsies were taken slightly more laterally than indicated in the original study by Terris et al. (9) so that the lateral aspects of the peripheral zone were covered. Transition-zone biopsies were not carried out. A seventh biopsy was directed toward hypoechoic lesions if applicable.

Pathologic Examination of Biopsy Specimens and Radical Prostatectomy Specimens

After routine fixation in a buffered 4% formalin solution, biopsy cores were embedded separately in paraffin blocks. Biopsy cores were longitudinally sectioned at three 5- μ m levels. Standard hematoxylin–eosin-stained histologic slides were prepared and examined histologically.

Radical prostatectomy specimens were fixed for 24 hours in a solution containing saline and buffered 4% formalin. After fixation, each specimen was step-sectioned at 4-mm intervals and totally embedded in paraffin blocks as described previously (10). From each paraffin block, standard hematoxylin–eosin-stained histologic slides were prepared for routine pathologic examination. Routine histopathologic examination included the determination of pathologic stage (tumor–lymph nodes–metastasis system [TNM], 1992) (11) and Gleason score (12). After histologic examination, all areas containing cancer were outlined on the slides. Gray-scale digital images of each histologic section were made with a digital camera, and then digital morphometric analysis was performed to measure each tumor area with the use of computer software for morphometry (Kontron Imaging System, model KS400; Kontron Elektronik GmbH., Eching, Germany). We determined tumor volume by adding all measured tumor areas and total slide areas (in millimeters squared) and multiplying them by 4 (the thickness in millimeters of the original slices). Earlier experiments had shown that no correction factor for the effects of fixation and paraffin embedding was required.

To assign a clinical importance to the tumors, we categorized all tumors as described previously (10). In brief, tumors that were smaller than 0.5 mL and confined to the prostate without harboring high-grade cancer (Gleason pattern 4 or 5) were classified as “minimal.” Prostate-confined tumors and well-differentiated tumors (no Gleason pattern 4 or 5) that showed capsule penetration were classified as “moderate.” All other tumors, including those that showed invasion of the seminal vesicles or bladder neck, were classified as “advanced.”

Statistical Evaluation

Detection rate is defined as the number of cancers found in participants who were screened in one round, in this case during the prevalence screen. Positive predictive values (i.e., the proportion of those with a positive test who are diagnosed with prostate cancer) were calculated as described previously (13). Sensitivity and specificity were estimated by use of prevalence estimates as explained below. The definitions given by Essex-Sorlie (13) were applied. Sensitivity can be calculated only if the underlying prevalence of the disease under discussion is known. This information is not available when screening for prostate cancer. To estimate the underlying prevalence of cancers detectable by

screening, we performed logistic regression analysis on screening data. This analysis results in estimating the number of men who would have been diagnosed to have prostate cancer by their PSA levels, if everyone had been subjected to a biopsy examination. We refer to this procedure as an *a priori* prevalence assessment (APPA). Clinical judgment suggests that, for each range of PSA levels, cancers predicted by an APPA would have the same characteristic volume and aggressiveness as those diagnosed, as long as identical biopsy procedures were used. APPA does not include cancers with a very small volume. Only small proportions of such cancers are detected because of the limited sampling by sextant biopsy.

The model applied is a simple logistic regression model using PSA, DRE, TRUS, and prostatic volume (TRUS estimated) as predictors for prostatic cancer. The model has been tested prospectively (Kranse R, Beemsterboer P, Rietbergen J, Habbema D, Hugosson J, Schröder FH: unpublished data). No interaction terms were added. These terms were studied but did not contribute significantly to the model. Validation was done by testing the model on a sample cohort from the Swedish partner in ERSPC (Göteborg). The model was shown to be applicable with remarkable accuracy to this independent population. The outcome of the model can be interpreted as the chance to detect prostate cancer in a sextant biopsy given the outcome of the screening tests and, therefore, can also be used to assess the chance to detect prostate cancer in those men who were not subjected to biopsy examination (extrapolation). In the model, the outcome given as the numbers of cancers found by the large number of biopsies done in men with normal DRE and PSA values of 4.0 ng/mL or more was used. These biopsies provide information about the value of PSA without a suspicious DRE and/or TRUS. This knowledge is included in the model and is used in the extrapolation to a PSA range that is less than 4.0 ng/mL with a negative DRE and/or a negative TRUS. In this way, the number of cancers that would have been found if all men were subjected to biopsy examination can be estimated (by APPA), and an estimate of sensitivity can be obtained. It must be kept in mind that the true number of tumors with roughly similar characteristics is underestimated in this way, because sextant biopsies miss relatively large tumors (14). Thus, the

sensitivities that we have calculated are probably still too high.

RESULTS

Overall Results of Screening (n = 10 523 Men)

The overall detection rate of prostate cancer when PSA level measurement, DRE, and TRUS are used is 4.5%. The positive predictive value is strongly dependent on the underlying prevalence of prostate cancer, which clearly depends on PSA levels. In the protocols used in this study, on average, 4.8 biopsies were necessary to detect prostate cancer. However, the positive predictive value of PSA levels between 0 and 0.9 ng/mL is extremely low, and the number of biopsies per cancer detected in individuals who have PSA values in this range is 183/4 or 46 biopsies. What is an acceptable number of biopsies done per cancer detected?

The Venn diagram presented in Fig. 2, A, is complementary to the information in Table 1. It presents the numbers of biopsies performed as the result of single and combined tests, the number of biopsies necessary to find one prostate cancer, and the number of cancers detected. Clearly, the performance improves whenever more than one test is positive. Positive predictive values for tests and test combinations independent of PSA levels can be calculated from this diagram by dividing the number of cancers by the number of biopsies. All participants whose data are included in these statistics were in fact subjected to biopsy examination if a biopsy was indicated by a PSA level of 4.0

ng/mL or more, a suspicious DRE, and/or a suspicious TRUS.

Predictive Value of DRE as a Function of PSA Range

Among the 8367 men who were in fact screened by all three tests, data for those who had a positive DRE and were screened by biopsy examination are shown in Table 1. Those men who also had an elevated PSA level or a suspicious TRUS result are included. Overall, 970 biopsies were used to diagnose 264 cancers (55.8% of all cancers) with a positive predictive value of 27%; 82 (17.3%) cancers would have remained undetected by PSA-based screening alone. We assumed that all participants who had an abnormal DRE and were found to have cancer would have been diagnosed with an abnormal DRE alone. This assumption seems justified because the same biopsy procedure was used for all participants with an elevated PSA, an abnormal TRUS, or an abnormal DRE.

With PSA values of 0–3.9 ng/mL, the positive predictive values range from 4% to 33%, with an average of 12.8%. Thus, 7.8 biopsies must be done to find one cancer. With PSA values of 0–2.9 ng/mL, the positive predictive values range from 4% to 11%, with an average of 8.8%. The situation changes drastically in those men whose PSA values are greater than or equal to 3.0 ng/mL. The positive predictive value increases to 49.6%, and two biopsies must be done to find one cancer. Detection rates, however, are dramatically lower with DRE alone and strongly depend on PSA levels. Thus, the perfor-

Table 1. Results of screening from the European Randomized Study of Screening for Prostate Cancer, Rotterdam section, from 1994 through 1997 in 10 523 men*

PSA level, ng/mL	PSA, TRUS, and DRE							
	No. screened (a)	No. of biopsies (b)	Prostate cancer		No. of needle biopsies (d)	DRE alone		
			No. (c)	Detection rate, %†		No. of prostate cancers (e)	PPV, % (e/d)	Detection rate, %†
0–0.9	1702	183	4	0.12	109	4	4	0.2
1.0–1.9	3305	502	43	1.3	296	29	10	0.3
2.0–2.9	1314	217	29	2.2	128	14	11	1.1
3.0–3.9	734	181	46	6.3	106	35	33	4.8
4.0–9.9	1095	988	238	21.7	249	115	45	10.5
≥10.0	217	196	113	52.1	82	67	83	30.9
Total	8367	2267	473	4.5	970	264	27	2.5

*Results of screening of 10 523 men are presented. The use of all three tests is compared with the use of DRE alone. Because of a change in the protocol in February 1996, men with PSA levels of 0–0.9 ng/mL were no longer screened by TRUS and DRE. A total of 2156 men with PSA values less than 1 ng/mL have entered the study since this time. PSA = prostate-specific antigen; TRUS = transrectal ultrasonography; DRE = digital rectal examination; PPV = positive predictive value.

†Detection rate = number of cancers/number of men screened (473/10 523 = 0.0449 and 264/10 523 = 0.025).

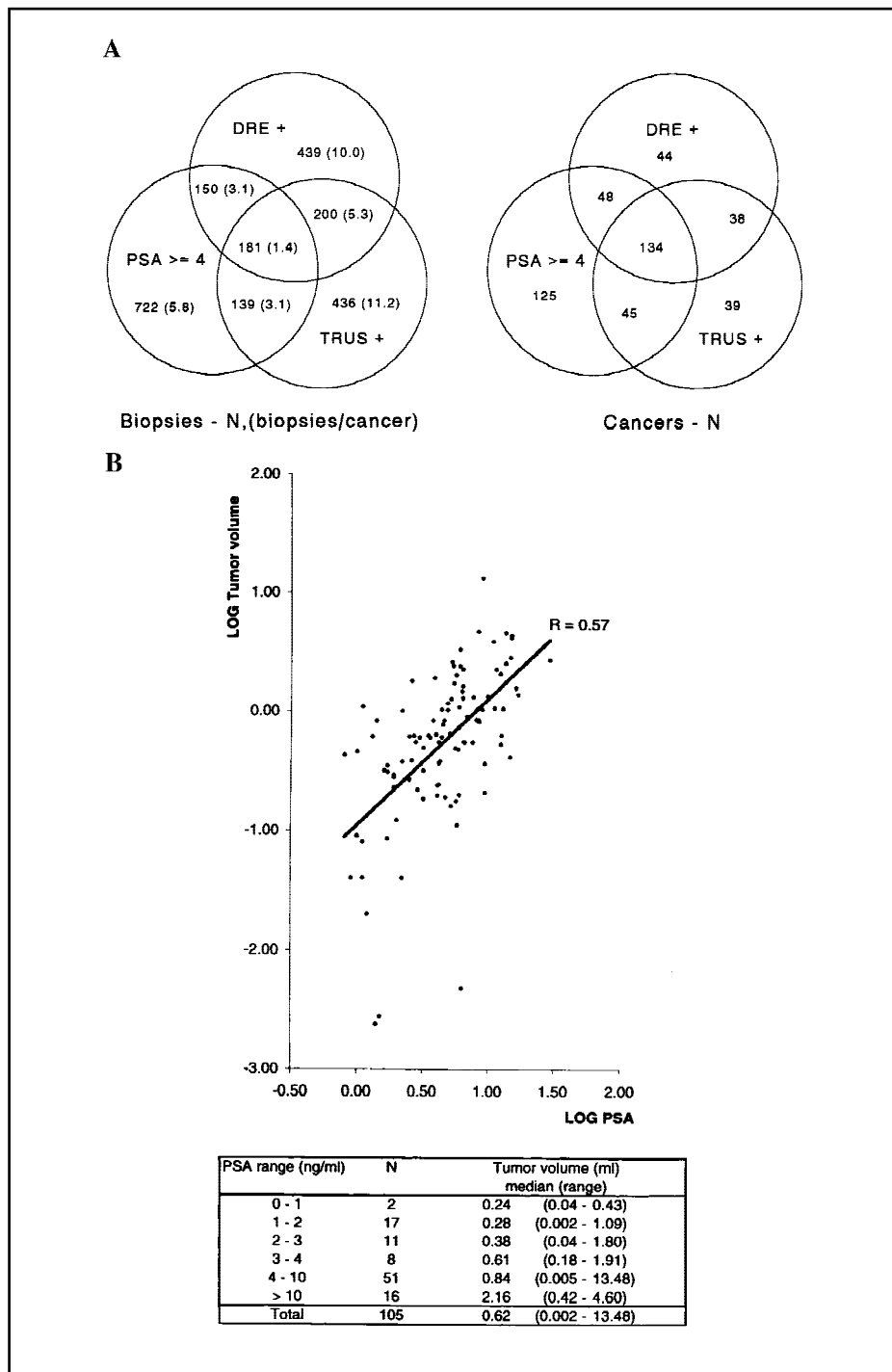


Fig. 2. A) Venn diagram of the results of screening by digital rectal examination (DRE), transrectal ultrasonography (TRUS), and prostate-specific antigen (PSA) in 10523 men randomly assigned to screening. Two thousand two hundred sixty-seven biopsies detected 473 cancers (detection rate = 4.5%). Among 3858 men with serum PSA levels of 0–0.9 ng/mL, only 1702 underwent DRE and TRUS because of a policy change in 1996. **B)** Correlation between serum PSA level and prostate cancer volume in 105 participants after radical prostatectomy.

mance of DRE is strongly dependent on PSA levels and is poorest when the PSA level is less than 3.0 ng/mL, an area that is considered the domain of the DRE.

Sensitivity and Specificity of DRE

Table 2 shows that the overall sensitivity and specificity of DRE are 37% and

91%, respectively. This table introduces the parameter APPA, an estimate of the underlying prevalence of prostate cancer as a function of the PSA level. The APPA is based on a logistic regression analysis that considers DRE, TRUS, PSA, and prostatic volume. The sensitivity of the DRE increases with increasing PSA lev-

els. The sensitivity of the DRE is 20% when the PSA level is less than 3.0 ng/mL. When the PSA level is 3.0 ng/mL or more, the sensitivity of the DRE becomes 46%. Sensitivity indicates the proportion of cancers detected by DRE of the cancers that would have been detected if every participant had been subjected to biopsy examination. Consequently, for the 1702 participants with PSA levels in the range of 0–0.9 ng/mL, 19 participants are assumed to have a cancer that could have been diagnosed with sextant biopsies (from the APPA), but only four of those cancers (21%) were detected by DRE followed by biopsy. With PSA values greater than or equal to 10.0 ng/mL, 128 of 217 participants in this group were expected to have prostate cancer with characteristics comparable to those of cancers that have been diagnosed. Sixty-seven (52%) of these 128 would be expected to have been diagnosed if DRE had been the only test used. The specificity remains greater than 83% over the total range of PSA values. The sensitivity data show a very low predictive capability for DRE in the low PSA ranges.

Tumor Volume and Grade

Roughly half of the patients whose cancers (230–240 cancers) were detected in this series have undergone radical prostatectomy. We have done complete morphometric and histologic analyses of tumor volumes and have determined the Gleason score for 105 of these tumors. In Table 3, the mean tumor volumes and Gleason scores and their ranges are listed separately by PSA level for 49 patients who had negative DRE results and for 56 patients who had positive DRE results. Tumor volumes show a weak but statistically significant correlation with PSA, which is also illustrated by Fig. 2, B. The correlation coefficient and two-sided *P* value are .57 and <.001, respectively. Very clearly, the smallest tumors are found in the very low PSA ranges, their tumor volume does not differ between men with positive DREs and negative DREs, and the volumes of most tumors are so small that detection by DRE is unlikely. If one considers palpable prostate cancer to be spherical, tumors with volumes of 0.02, 0.03, 0.07, and 0.5 mL would have diameters of 0.3, 0.4, 0.5, and 1.0 cm, respectively. Of 38 tumors from patients with PSA values in the range of

Table 2. Sensitivity and specificity of digital rectal examination (DRE)*

PSA level, ng/mL	No. of DREs (a)	No. of APPAs (b)	Biopsy		Sensitivity (d/b)	Specificity, % [(a - c) - (b - d)] / (a - b)
			No. (c)	No. of PCs (d)		
0-0.9	1702	19	109	4	21	94
1.0-1.9	3305	119	296	29	24	92
2.0-2.9	1314	97	128	14	14	91
3.0-3.9	734	89	106	35	39	89
4.0-9.9	1095	254	249	115	45	84
≥10.0	217	128	82	67	52	83
Total	8367	706	970	264	37	91

*We screened 8367 men by use of the DRE and found that 970 men required biopsy examination. These biopsy examinations detected 264 prostate cancers (PCs). The *a priori* prevalence assessment (APPA) of prostate cancer as a function of prostate-specific antigen (PSA) level was used. The logistic regression model applied for determining APPA uses PSA, DRE, transrectal ultrasonography, and prostatic volume. These data give an estimate of the number of cancers that would have been found if all patients had received a biopsy examination.

0-3.9 ng/mL, 16 tumors (42%) showed the characteristics of minimal cancer, whereas no advanced cancer was present. Of 67 tumors from patients with PSA levels of 4.0 ng/mL or more, the numbers of minimal, moderate, and advanced tumors were seven (10%), 32 (48%), and 28 (42%), respectively. Of those with PSA values of less than 4.0 ng/mL, 16 (42%) were minimal cancers, 16 (42%) were moderate cancers, and six (16%) were advanced cancers. Of all 105 cancers, 22%, 51%, and 16% were classified as minimal, moderate, and advanced, respectively.

DISCUSSION

The usefulness of DRE in screening for prostate cancer is the main issue discussed in this report. Prior to the advent of PSA and TRUS, DRE was the only available screening test for prostate cancer. In clinical use, 40%-50% of all palpable abnormalities that were suspected of being prostate cancer, if subsequently subjected to biopsy examination, were found to be malignant (15). Such data cannot be ap-

plied to population-based screening. DRE is a strongly investigator-dependent test that requires time to master. In addition, the predictive value of the screening tests will be different in patients presenting with symptoms as opposed to the general population.

Since the decision to perform a biopsy in patients with PSA values of 3.0 ng/mL or more or 4.0 ng/mL is often driven by the results of the PSA test, we have concentrated our efforts on the lower PSA ranges, where DRE is the mainstay of early diagnosis.

A review of the older literature (16) describes an average detection rate with DRE alone of 0.85% and a positive predictive value of 28%. Seventy-three percent of all cancers were detected in a clinically locally confined state. A number of older studies predating the PSA era and more recent reports deal with the usefulness of DRE as a screening test alone and in combination with other tests (17-24).

In spite of a very large number of participants and cancers already detected and

in spite of our knowledge of tumor characteristics, definitive judgments on the value of individual screening tests or screening algorithms can be made only after the results of the definitive randomized screening studies are available. Statistical correlation with the main end point, which is prostate cancer mortality, will be of crucial importance. Also, data on important aspects of quality of life (such as difficulties with the screening process itself), the diagnostic and biopsy procedures, and cost are not available and cannot be judged at this time.

Predictive Value of DRE

Our data indicate that the DRE has a low positive predictive value in men with low PSA levels. This result is due to the low prevalence of the disease and the low sensitivity of the screening procedure. When PSA levels are less than 3.0 ng/mL, 11 biopsies are necessary to detect one cancer. If it were certain that those cancers detected in this PSA range pose a significant threat to the patient's life, this burden might be acceptable (however, many of these cancers do not seem to pose such a threat). Selective screening for potentially aggressive lesions is important. Many prostate cancers detected in men with PSA levels of less than 4.0 ng/mL do not show the characteristics of aggressive disease by volume and grade of differentiation. A large proportion of the tumors detected may not be immediately clinically relevant and would probably be detected through subsequent screening, as suggested by Carter et al. (25). Since a PSA level of less than 4.0 ng/mL does not indicate the need for a biopsy, for PSA levels less than 4.0 ng/mL in this study, one might expect that cancers found by

Table 3. Tumor volumes and Gleason scores as a function of prostate-specific antigen (PSA) level and digital rectal examination (DRE) results (n = 105)

PSA range, ng/mL	DRE-negative result*			DRE-positive result‡		
	No. of DRE-negative cases	Tumor volume, mL†	Gleason score	No. of DRE-positive cases	Tumor volume, mL†	Gleason score
0-0.9	0			2	0.24 (0.04-0.43)	6 (6-6)
1.0-1.9	4	0.11 (0.002-0.23)	6 (5-7)	13	0.36 (0.003-1.09)	6 (5-7)
2.0-2.9	3	0.49 (0.22-0.62)	7 (7-7)	8	0.57 (0.04-1.80)	7 (6-7)
3.0-3.9	4	0.57 (0.32-0.84)	7 (7-7)	4	0.83 (0.18-1.91)	7 (7-7)
≥4.0	38	0.96 (0.005-4.34)	6.5 (4-8)	29	2.22 (0.16-13.48)	7 (5-9)
Total	49	0.83 (0.002-4.34)	7 (4-8)	56	1.38 (0.003-13.48)	7 (5-9)

*A DRE-negative result is defined as nonpalpable cancer.

†Data are the mean with the range in parentheses.

‡DRE-positive result is defined as palpable cancer.

DRE and/or TRUS are larger, clearly palpable, and visible tumors that do not produce much PSA, a feature often associated with poor differentiation. The contrary, however, is the case.

To improve the understanding of this situation further, the sensitivity of DRE as a function of PSA level was calculated by use of an estimate of the *a priori* prevalence of prostate cancer obtained by logistic regression modeling (APPA). The proportion of these potentially diagnosable cancers, diagnosed by DRE, remains very low, perhaps because many of these tumors are too small to be palpable by DRE. In Table 2, sensitivity in this context indicates the number of cancers detected relative to those that would have been detected if every participant had been subjected to biopsy examination. A screening test that identifies only 20% of identifiable cancers has a very low predictive value. Consequently, for this test, the philosophic and ethical problem then is to determine the lower limit of the sensitivity that is acceptable. If screening could be made selective enough so that aggressive tumors could be identified with reasonable accuracy and so that non-aggressive lesions could be eliminated from immediate treatment, a lower sensitivity might be acceptable than in the present situation. The slight decrease of the specificity with increasing levels of PSA remains unexplained but could be related to benign prostatic hyperplasia. The presence of precursor lesions may be another explanation. The positive predictive value of the test combination overall is $473/2267 = 21\%$, and the sensitivity (Table 2) is 37%. This result compares unfavorably with the findings in regard to mammography in the Dutch National Screening trial, which had a positive predictive value of 45.4% with 2.2 biopsies per cancer detected (26). In the German mammography study, the sensitivity of mammography and physical examination was overall 73% (27). This is in line with the 68% sensitivity reported in the Health Insurance Plan trial in the United States (28).

As far as the ongoing screening study is concerned, the predictive value of a screening test has to be viewed in a different context as long as it remains uncertain which tumors will progress rapidly and which tumors will not. The final aim of the study, namely, to show or to ex-

clude a difference in prostate cancer mortality, may be jeopardized by various exclusions. Thus, a change in screening strategies within protocols requires careful exclusion of the possibility that relevant tumors are eliminated from a given study. Still, with the combination of volume and grade of differentiation being likely, but for the most part unproven, prognostic parameters in this respect, the risk of missing potentially aggressive tumors remains.

Tumor Aggressiveness

Men with prostate cancer who have PSA levels, measured at an initial screening, of less than 3.0 ng/mL usually harbor prostate cancer that has a favorable prognosis. However, definitions of what might be nonaggressive disease have not been tested prospectively and are likely not to be applicable to the individual patient. Furthermore, the biologic potential of individual lesions may be unpredictable. It is a disturbing finding that, in our series of carefully studied radical prostatectomy specimens, up to 60% of cases contained poorly differentiated foci (Hoedemaeker RF, Rietbergen JB, Kranse R, van der Swast TH, Schröder FH: unpublished data). Without being able to correlate these findings to important end points, such as prostate cancer mortality, it will be impossible to determine exactly what may be a clinically unimportant prostate cancer or a tumor with a minimal risk that justifies a delay in diagnosis and treatment. Even more difficult will be the identification of such cancers on biopsy specimens because of the well-documented difficulty of identifying poorly differentiated disease. A study (29) indicated that undergrading with respect to Gleason scores 7–10 may be in the range of 50%. To develop biopsy techniques that are more representative of tumor composition and to improve judgment on aggressiveness are top research priorities in this field. Another source of uncertainty lies in the fact that only about half of the patients with diagnosed cancers in this study underwent radical prostatectomy, and selection bias cannot be excluded.

How to Continue?

This study presents evidence that the DRE has a very low predictive value with

respect to diagnosing prostate cancer in men with PSA levels of less than 3.0 ng/mL and in large proportions of men with PSA levels between 3.0 and 3.9 ng/mL. Large numbers of biopsies are necessary to diagnose small numbers of tumors. This can be considered an inherent feature of screening. However, cancers found often have the characteristics of clinically nonaggressive tumors, and these cancers at the time of diagnosis are probably not life-threatening. In addition, the screening procedure is bothersome and not without danger for the participants. If at all possible, in those men who have PSA levels of less than 3.0 or 4.0 ng/mL, the DRE should be replaced with a more sensitive test. Research should concentrate on alternative technologies and algorithms. The predictive power of using the free/total ratio of PSA in men with low total PSA values has been insufficiently explored, but its use may be important to improve specificity and selectiveness of screening (30). Also, experimental confirmation of the correctness of model predictions of the underlying prevalence of prostate cancers needs to be obtained.

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NOTES

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Effect of BRCA1 and BRCA2 on the Association Between Breast Cancer Risk and Family History

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Background: The discovery of BRCA1 and BRCA2 has led to a reassessment of the association between family history of breast/ovarian cancer and breast cancer risk after controlling for carrier status for mutations in the BRCA1 and BRCA2 genes. We examined whether family history of breast cancer remains a predictive risk factor for this disease after carrier status for BRCA1 and/or BRCA2 mutations is taken into consideration. **Methods:** The data are from 4730 case subjects with breast cancer and 4688 control subjects enrolled in the Cancer and Steroid Hormone Study. The probability of being a BRCA1 and/or BRCA2 gene carrier was calculated for each woman. Among predicted noncarriers, logistic regression was used to assess the relationship (odds ratios and 95% confidence intervals [CIs]) between case or control status and family history of breast or ovarian cancer. Estimates of age-specific breast cancer risk are presented by predicted carrier status. **Results:** Among predicted noncarriers, case subjects were 2.06 times (95% CI = 1.69–2.50) and 1.24 times (95% CI = 1.17–1.32) more likely to report a first-degree or second-degree family history of breast cancer, respectively, than were control subjects. Case subjects were 1.99 times (95% CI = 1.63–2.44), 1.66 times (95% CI = 1.18–2.38), and 2.23 times (95% CI = 0.21–24.65) more likely to report an affected mother, sister, or both, respectively, than were control subjects. A family history of ovarian cancer was not statistically significantly associated with breast cancer risk. Noncarriers were predicted to have a lifetime risk of 9% of developing breast cancer compared with a 63% risk for carriers. **Conclusions:** Among women with a moderate family history

of breast cancer, i.e., predicted noncarriers of BRCA1 and/or BRCA2 mutations, family history remains a factor in predicting breast cancer risk. In families with breast and ovarian cancers, the aggregation of these two cancers appears to be explained by BRCA1/BRCA2 mutation-carrier probability. [J Natl Cancer Inst 1998;90:1824–9]

It is well established that a family history of breast cancer is associated with an increased risk of developing breast cancer (1–10). In fact, among those variables that have been shown to bear a relationship with breast cancer, the greatest increase in risk, after controlling for age, has generally been associated with the presence of a positive family history of breast cancer (1–10). Published statistical estimates of the proportion of breast cancer in the general population that is likely to be attributable to an inherited mechanism range from approximately 6% to 19% (1–3,11–17), depending on the type of relative included in the calculation. When based solely on information obtained from first-degree relatives, this risk is widely estimated to be approximately 6%–7% (2,3,11), averaged across all ages at onset. The recent discovery of two genes associated with the development of breast cancer, BRCA1 and BRCA2 (18–25), along with preliminary laboratory-based prevalence data for these genes, allows investigators to begin to refine statistical estimates of the familial attributable risk of breast cancer.

At present, data suggest that a mutation in BRCA1 accounts for the majority (80%–90%) of families containing multiple case subjects with breast and/or ovarian cancer and approximately 45% of inherited breast cancer (12), whereas a mutation in BRCA2 is thought to account for approximately 35% of inherited breast cancer (21,22). Despite explaining a high proportion of breast and/or ovarian cancer incidence in high-risk families, current prevalence data on mutations in BRCA1 and BRCA2 genes indicate that the vast majority of women as well as the majority of case subjects with breast cancer in the United States are not carriers of mutations in these genes. Furthermore, most women with a family history of breast cancer are not members of high-risk families for breast and/or ovarian cancer, but instead

have one or perhaps two family members affected with breast cancer. Therefore, the extent to which a positive family history of breast cancer remains a factor in the prediction of breast cancer risk outside high-risk families and after the estimated effects of mutations in BRCA1 and BRCA2 genes have been taken into account remains an important issue.

This report will examine whether a role for family history remains as a predictive risk factor for breast cancer once the effects of BRCA1 and BRCA2 have been taken into account.

SUBJECTS AND METHODS

Data were obtained from the Cancer and Steroid Hormone Study, a multicenter, population-based, case-control study conducted by the Centers for Disease Control and Prevention. The dataset consists of 4730 case subjects aged 20–54 years with histologically confirmed breast cancer and 4688 control subjects. The case subjects were registered between December 1, 1980, and December 31, 1982, at eight Surveillance, Epidemiology, and End Results (SEER)¹ Centers of the National Cancer Institute. Control subjects were selected through random-digit dialing and were matched by geography and 5-year age intervals to the case subjects. The eight centers include the cities and metropolitan areas of Atlanta (GA), Detroit (MI), San Francisco (CA), and Seattle (WA); the four urban counties of Utah; and the states of Connecticut, Iowa, and New Mexico. Case subjects with a history of breast cancer or a breast biopsy of unknown outcome were excluded from the study. In-home interviews were used to collect information on a wide variety of covariates for each of the case subjects and control subjects, including menstrual and pregnancy histories, use of oral contraceptives, and history of benign breast disease. In addition, case subjects and control subjects were interviewed about the occurrence of cancer in specific first-degree and second-degree female relatives. Cancer history in male relatives was not collected. A detailed description of the study may be found elsewhere (26).

The probability of carrying a mutation in BRCA1 or BRCA2 or both is calculated for each case subject and control subject (i.e., proband) by use of Bayes

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theorem and Mendelian genetics, assuming an autosomal dominant transmission for both BRCA1 and BRCA2 genes (27,28). In each instance, a woman's probability of being a gene carrier is calculated conditional on the breast and ovarian cancer status of her first-degree and second-degree female relatives and the age at onset of any affected female relatives as well as the current age or age at death of any unaffected female relatives. The calculation of carrier probability also takes into account whether or not the proband identified herself as Jewish in the original questionnaire. The model uses BRCA1 and BRCA2 prevalence estimates from Ford et al. (14) as well as penetrance estimates from Easton et al. (12) for women who did not identify themselves as Jewish and from Struwing et al. (29) for women who did identify themselves as Jewish. The calculation of carrier probability does not include the cancer status of the proband because this is the outcome we wish to predict using an individual's family history profile. If M represents "individual carries a BRCA1/BRCA2 mutation" and H represents "individual's family history," then by Bayes theorem,

$$P(M/H) = [P(H/M) \times P(M)]/[P(H)]. \quad [1]$$

The details of this model have been described previously (27,28).

By use of this model, women with a joint probability of less than or equal to 1% of carrying mutations in either BRCA1 or BRCA2 genes were defined as noncarriers. In this analysis, first-degree family history was defined as a mother or sister affected with breast cancer. Second-degree family history was defined as a grandmother or aunt affected with breast cancer. Study subjects were defined to have unknown family history if the breast cancer status of all first-degree relatives was unknown. The initial portion of the statistical analysis included descriptive statistics. The association between the risk of breast cancer and independent covariates was examined by t tests and chi-squared tests. To assess the relative risk of breast cancer associated with a positive family history of breast cancer in women unlikely to carry mutations in BRCA1 or BRCA2, logistic regression was used on predicted noncarriers to provide maximum likelihood estimates of the odds ratios (ORs) (adjusted and unadjusted) with 95% confidence intervals (CIs) by use of the statistical package PC-SAS version 6.11 (30). Women with affected first-degree relatives only, affected second-degree relatives only, or both affected first-degree and second-degree relatives were compared, respectively, with women with no affected relatives. The risks are adjusted for BRCA1 and BRCA2 mutation carrier probability and any significant (at a 5% type I error level) environmental covariates, including age (years), race (white/black), parity, age at first live birth (years), menopausal status (premenopausal/postmenopausal), and history of benign breast disease (yes/no).

To estimate the age-specific and cumulative risk of developing breast cancer among probable carriers and probable noncarriers, we divided case subjects and control subjects (i.e., the probands) into the following two groups by carrier probability: 1) noncarriers (defined as a proband having a carrier probability of 0.00–0.01) and 2) carriers (defined as a proband having a carrier probability of 0.70–0.99). The two probability groupings were selected in an effort to maintain relatively homogeneous groups

while containing sufficient numbers of relatives at risk. For this analysis, carrier probabilities were recalculated to incorporate proband cancer status (i.e., whether the proband was herself affected and the age at onset or current age for the proband). This was done because this portion of the analysis focuses on risk to relatives rather than on risk to proband. The observed age-specific Kaplan–Meier risks of breast cancer in mothers and sisters of the probands were then computed. The analysis is done under the assumption that relatives of noncarriers are themselves likely to be noncarriers; hence, the risks associated with these women can be seen to represent those of the general noncarrier population. Among first-degree relatives of women predicted to be carriers of BRCA1 and/or BRCA2 mutations, approximately 50% would be themselves expected to be carriers under an autosomal dominant genetic model, whereas the remaining 50% would be expected to be normal homozygotes. (We assume that the homozygotes with mutations in both BRCA1 and BRCA2 genes are extremely rare and therefore not included in any calculations.) The observed Kaplan–Meier risk estimates for breast cancer seen for relatives of putative mutation carriers represent an average across the two groups of relatives. Therefore, the age-specific risks of breast cancer to carriers, R_{carriers} , may be estimated as twice the Kaplan–Meier risks calculated among first-degree relatives of putative carriers, $R_{0.70-0.99}$, minus the value for first-degree relatives of putative noncarriers, $R_{0.00-0.01}$ (11,29).

$$R_{0.70-0.99} = (R_{\text{noncarriers}} + R_{\text{carriers}})/2 \quad [2]$$

$$\text{if } R_{\text{noncarriers}} = R_{0.00-0.01} \text{ then}$$

$$2 \times R_{0.70-0.99} - R_{0.00-0.01} = R_{\text{carriers}}. \quad [3]$$

RESULTS

Women with a joint probability less than or equal to 1% of carrying mutations in either BRCA1 or BRCA2 genes are defined as noncarriers. By use of this definition, 4337 (91.7%) of 4730 case subjects and 4447 (94.9%) of 4688 control subjects were predicted to carry neither BRCA1 nor BRCA2 mutations. Among case subjects diagnosed at the ages of 20–29 years, 10.5% were predicted to be carriers of either BRCA1 or BRCA2 mutations. This number decreased to 7.5% among women diagnosed between the ages of 50 and 54 years. Among women defined as noncarriers, the mean probability of being a BRCA1 mutation carrier was estimated at 6.8×10^{-4} , with a range from 6.41×10^{-6} to 9.8×10^{-3} . The mean probability of being a BRCA2 mutation carrier was estimated at 3.2×10^{-4} , with a range from 2.4×10^{-9} to 6.5×10^{-3} . Twenty-five percent of noncarriers versus 66% of carriers reported a family history of breast cancer. Approximately 2.2% of noncarriers versus 33% of carriers have a family history of ovarian cancer. The ma-

majority of women predicted to be noncarriers and who report a family history of breast cancer have a single first-degree or second-degree relative affected with breast cancer. This relative in general was younger in age for the carrier group than for the noncarrier group (44 years versus 56 years). Among women with both a mother and sister affected with breast cancer, the mean age of affected relatives for carriers versus noncarriers was calculated at approximately 46 years versus 62 years, respectively. For the remainder of this section, the terms "case subjects" and "control subjects" refer to the 4337 case subjects and 4447 control subjects defined as noncarriers.

Case subjects and control subjects did not differ with respect to religion, race, parity, or number of sisters. Case subjects were more likely than control subjects to be older (44.4 years compared with 43.8 years), younger at menarche, older at first live birth, premenopausal, and to have a history of benign breast disease.

Case subjects were 2.06 times (95% CI = 1.69–2.50) and 1.24 times (95% CI = 1.17–1.32) more likely to report a first-degree or second-degree family history of breast cancer, respectively, than were control subjects. These numbers did not change significantly when the model was adjusted for mutations in BRCA1 and BRCA2 gene carrier probability (both of which were nonsignificant) (Table 1) or for age, menopausal status, history of benign breast disease, and age at first full-term pregnancy (data not shown). Although a positive family history of breast cancer was significantly related to breast cancer risk among noncarriers, the same was not true for the relationship between a positive family history of ovarian cancer and breast cancer risk. Case subjects with breast cancer were 1.43 times (95% CI = 0.85–2.43) and 0.99 times (95% CI = 0.81–1.20) more likely to report a first-degree or second-degree relative with ovarian cancer than were control subjects. In fact, when BRCA2 and BRCA1 mutation carrier probabilities were included as covariates in the model (to obtain adjusted ORs), family history of ovarian cancer had no significant role in predicting case or control status.

The risk of breast cancer by type of first-degree relative affected with breast cancer is presented in Table 2. Case subjects were 1.99 times (95% CI = 1.63–

Table 1. Risk of breast cancer in women unlikely to carry mutations in BRCA1 or BRCA2 genes, stratified by family history of breast cancer

Family history*	Odds ratio (95% confidence interval)	Adjusted odds ratio† (95% confidence interval)
None	1.0 (referent)	1.0 (referent)
First-degree only	2.06 (1.69–2.50)	1.90 (1.44–2.51)
Second-degree only	1.24 (1.17–1.32)	1.21 (1.13–1.29)
First-degree and second-degree	1.24 (1.09–1.42)	1.10 (0.91–1.33)

*Excludes 629 case subjects and 574 control subjects with unknown first-degree family history of breast cancer.

†Adjusted for BRCA1 and BRCA2 carrier probability.

Table 2. Risk of breast cancer in women unlikely to carry mutations in BRCA1 or BRCA2 genes, stratified by first-degree family history of breast cancer

Family history*	Odds ratio (95% confidence interval)	Adjusted odds ratio† (95% confidence interval)
None	1.0 (referent)	1.0 (referent)
Mother	1.99 (1.63–2.44)	1.55 (1.21–1.99)
Sister	1.66 (1.18–2.38)	1.21 (0.72–1.65)
Mother and sister	2.23 (0.21–24.65)	1.11 (0.09–13.77)

*Excludes 629 case subjects and 574 control subjects with unknown first-degree family history of breast cancer.

†Adjusted for BRCA1 and BRCA2 carrier probability.

2.44) and 1.66 times (95% CI = 1.18–2.38) as likely as control subjects to report an affected mother or sister, respectively. Women with both an affected mother and sister were at 2.23 times (95% CI = 0.21–24.65) the risk of developing breast cancer relative to women without such a family history, although there were only two case subjects and one control subject with such a family history. These values did not change significantly when adjusted for BRCA1 and BRCA2 carrier probability or for the above-mentioned environmental covariates, although the adjusted OR for women with both a mother and a sister affected is approximately half that of the unadjusted OR most likely due to instability of the estimate secondary to small sample size.

Case subjects diagnosed at ages 20–29 years, 30–39 years, 40–49 years, and 50–54 years were 5.30 times (95% CI = 0.94–29.75), 2.72 times (95% CI = 1.62–4.73), 2.13 times (95% CI = 1.59–2.86), and 1.70 times (95% CI = 1.24–2.32), respectively, more likely than control subjects to report a first-degree family history. These numbers did not differ significantly when adjusted for BRCA1 and BRCA2 mutation carrier probability with the exception of the age category 20–29 years for which small numbers (four case subjects and two control subjects with a positive family history) and multicol-

linearity among the three variables (family history, BRCA1 and BRCA2 mutation carrier probability) prevent calculation of an adjusted OR. The risk of breast cancer by age at onset and laterality of affected first-degree relatives, neither of which was a significant risk factor, is presented in Table 3.

The estimated age-specific and cumulative risks of breast cancer for carriers and noncarriers are presented in Table 4. For noncarriers, i.e., first-degree relatives of probands with joint carrier probability of 0.00–0.01, the estimated risks match those generally reported for the U.S. female population, especially for the years (1980–1982) during which these data were collected. As expected, the estimated

Table 3. Risk of breast cancer by age at onset and laterality of breast cancer in relatives of women unlikely to carry BRCA1 or BRCA2

	Odds ratio (95% confidence interval)	Adjusted odds ratio† (95% confidence interval)
Age at onset in first-degree relatives, y*		
None	1.0 (referent)	1.0 (referent)
≤45	2.84 (1.84–4.50)	2.00 (0.99–4.08)
>45	1.96 (1.47–2.62)	1.92 (1.54–2.39)
Laterality of cancer among first-degree relatives*		
None	1.0 (referent)	1.0 (referent)
Unilateral	2.21 (1.80–2.73)	1.94 (1.45–2.60)
Bilateral	1.19 (0.68–2.08)	1.10 (0.56–2.17)

*Excludes 629 case subjects and 574 control subjects with unknown first-degree family history of breast cancer.

†Adjusted for BRCA1 and BRCA2 carrier probability.

Table 4. Estimated cumulative risk, % (standard error, %) of breast cancer for predicted carriers and noncarriers of mutations in BRCA1/BRCA2 genes

Age at onset, y	Estimated cumulative risk, % (standard error, %)	
	Noncarriers	Carriers
0–29	0.0 (0.00)	4.3 (1.3)
30–39	0.2 (0.03)	19.8 (2.7)
40–49	0.8 (0.08)	41.3 (3.7)
50–59	1.6 (0.13)	50.4 (4.1)
60–69	2.7 (0.20)	52.8 (4.3)
70–79	5.1 (0.40)	62.8 (6.9)
≥80	8.8 (1.80)	62.8 (6.9)

rates for carriers (calculated as twice the Kaplan–Meier estimates for first-degree relatives of probands with joint carrier probability of 0.70–0.99 minus the Kaplan–Meier estimates for first-degree relatives of probands with joint carrier probability of 0.00–0.01) are much higher at all ages and predict a very high penetrance for women who carry mutations in BRCA1 or BRCA2 genes.

DISCUSSION

The extent to which the development of breast cancer may be attributed to inherited mutations in BRCA1 and BRCA2 genes remains a research area of intense investigation. Statistical and laboratory-based estimates of both carrier rates for such mutations and population attributable risk are being accrued in samples of affected and unaffected women. Using data from the Cancer and Steroid Hormone Study (CASH) in a previous analysis, Claus et al. (11) estimated the proportion of breast cancer attributable to inherited autosomal dominant genes to be approximately 33% of case subjects aged 20–29 years. This estimated risk de-

creased with age at onset to approximately 1.5% of case subjects aged 70 years or more. Using data from the Breast Cancer Consortium, Ford et al. (14) estimated that, in the general population, the proportion of breast cancer due to mutations in BRCA1 is 5.3% below the age of 40 years, 2.2% between the ages of 40 and 49 years, and 1.1% between the ages of 50 and 70 years. A third analysis (15), which combines data from three population-based, case-control studies of ovarian cancer (including the Cancer and Steroid Hormone Study), reports the proportion of case subjects with breast cancer due to BRCA1 and BRCA2 mutations to be 3.0% overall with a high of 11.2% among case subjects under the age of 30 years.

New laboratory data indicate that the proportion of breast cancer associated with BRCA1 may be higher than initially predicted by the Breast Cancer Consortium data but lower than that predicted by the CASH analyses (31–39). A study (32) of 80 women in whom breast cancer was diagnosed before the age of 35 reported that approximately 10% of these women carried germline alterations in the BRCA1 gene, whereas a second study (33) reported a mutation rate of 13% in a group of case subjects with breast cancer diagnosed before the age of 30. An analysis of women attending clinics that evaluate breast cancer risk (40) revealed a 7% rate of BRCA1 mutation in families with breast cancer but no ovarian cancer. Specific germline BRCA1 mutations, particularly the 185delAG mutation, have been identified at even higher rates among subsets of the general population, in particular young women or women of Ashkenazi Jewish background. Researchers have reported a 1% overall rate and a 21% prevalence rate among Jewish women diagnosed with breast cancer before the age of 40 (33–35). Similarly, a frequent germline BRCA2 mutation (6174delT) has also been estimated at approximately 2.7% in case subjects with early onset of breast cancer (41), 1% in the Ashkenazi Jewish population (36), and approximately 8% in Ashkenazi case subjects with breast cancer diagnosed before the age of 42 years (37). New data collected with the use of intensive sequencing techniques reveal strikingly high carrier rates in a collection of women diagnosed with ovarian cancer or early onset breast can-

cer (40). In this series, 31% of women affected with unilateral breast cancer before age 50 and with at least one affected relative were found to carry either BRCA1 or BRCA2. Women with more extensive family history had even higher rates.

In general, a positive family history of breast cancer has been associated with a twofold to threefold increase in the risk of developing breast cancer. Previous analyses of these data (9), calculated before the discovery of BRCA1 and BRCA2 and using the entire dataset, reported that women with a first-degree or second-degree relative with breast cancer had relative risks of 2.3 and 1.5, respectively, compared with the adjusted risks of 2.0 and 1.2 reported here for the subset of women predicted to be noncarriers of BRCA1 or BRCA2. Women with both a mother and sister affected had a relative risk of 14 compared with 2.3 here. For all combinations of family history, the risks are reduced when predicted noncarriers are examined, markedly so for women with multiple affected relatives, although the CI in this instance is wide and actually includes the value reported by Sattin et al. (9). This reduction is expected in light of the fact that these women are more likely to be either BRCA1 or BRCA2 carriers. In these data, the majority (92%) of women who reported both a mother and at least one sister affected with breast cancer were predicted to have an increased probability of being carriers of mutations in BRCA1 or BRCA2 genes and hence were excluded from the analyses. As would be predicted by the model, noncarriers (i.e. the remaining 8% of women) with both a mother and sister affected with breast cancer were more likely to have relatives affected at older ages than were carriers, with the mean age of affected relatives for carriers compared with noncarriers calculated at approximately 46 years versus 62 years, respectively. In these data, the majority of women predicted to be noncarriers and who report a family history of breast cancer have a single first-degree or second-degree relative affected with breast cancer. Once again, this relative is younger for carriers than for noncarriers (44 years versus 56 years). It is interesting to note that even this relatively moderate family history of breast cancer remains significantly associated with breast cancer risk, despite the fact that most of these

women are unlikely to carry either BRCA1 or BRCA2. A family history of ovarian cancer, however, appears to add no information to risk prediction once BRCA1 and BRCA2 carrier probability is known, matching existing laboratory data (42).

The estimated age-specific risks of breast cancer presented here for putative BRCA1 and BRCA2 mutation carriers compare reasonably well with those reported by other researchers (12–14,29,42), although the estimated lifetime risk of breast cancer reported here is relatively low. This appears to be due to the small numbers of older relatives among women predicted to be carriers (and the fact that probands in these data were from 20 to 54 years of age) and hence the presence of few affected older relatives from whom to obtain parameter estimates. A lifetime breast cancer risk of 63% for carriers in these data is compared with previously reported lifetime risks that range from 71% to 88%, depending on mutation type (12–14,42). As would be expected given our inclusion of published penetrance estimates (12,29) as model parameters, our risk estimates are intermediate between those of Struewing et al. (29) and Easton et al. (12).

There are multiple interpretations for the results presented in this report, which include a variety of genetic, environmental, or statistical sources of variation. Genetic explanations include the fact that 1) mutations in noncoding regions of BRCA1 or BRCA2 or 2) other inherited, as yet unidentified, breast cancer genes, in addition to BRCA1 and BRCA2, may account for some portion of association between family history and breast cancer risk. Although BRCA1 and BRCA2 appear to explain the majority of inherited early onset breast cancer, a number of families with large numbers of case subjects with early onset breast cancer have been shown to be unlinked to BRCA1 and BRCA2 (16,21,25,39,43). Additional genes have already been implicated in the development of breast cancer (44–47); one study (44) associated one in 11 cancers of the breast in the general population with rare alleles of a minisatellite locus adjacent to the HRAS1 gene located on chromosome 11. In addition, the p53 gene has also been associated with the development of breast cancer in families characterized by the Li-Fraumeni syn-

drome (45). The extent to which germline mutations in BRCA1, BRCA2, and other breast cancer susceptibility genes (47,48) play a role in the expression of breast cancer will be determined as population-based screening continues.

The observed correlation of breast cancer status within families may also be explained by familial aggregation of environmental risk factors; i.e., family history may serve as a proxy variable for environmental factors such as socioeconomic status, diet, and age at first live birth that are correlated within families and that are themselves risk factors for the development of breast cancer. Correlations in breast cancer risk factors, such as age at menarche, with correlation coefficients of .2-.5, .25-.4, and .18-.65 have been reported, between random pairs of mothers and daughters, between sisters, and between twins, respectively (49). In addition, statistically significant associations in age at first pregnancy and age at menarche between related women have been reported (49). However, simulation studies (50,51) have shown that, even with complete correlation in exposure among family members, environmental variables must have relatively high values for relative risk for disease, i.e., on the order of 10-fold, to lead to even modest increases in recurrence risk among family members. Since the majority of environmental risk factors for breast cancer have been associated with increases on the order of twofold or less, it seems unlikely that simple familial clustering of these factors could entirely explain all of the remaining familial aggregation in breast cancer seen here, although it is likely to play some part in that aggregation.

In these data, accurate calculation of carrier probability depends on a correctly specified statistical model as well as on correct estimates of penetrance and prevalence. Two groups of independent investigators (52,53) have undertaken validation studies of the carrier probability model used here. Both have reported a good overall predictive ability in identifying the presence of a mutation at either gene. Efforts are under way for a systematic multicenter validation of the model based on tested families. Statistical methodology and preliminary results are discussed by Iversen et al. (54). There are initial indications that the currently used values of penetrances and prevalences

may lead to underestimating carrier probabilities for weak family histories. This is consistent with the belief that the penetrance functions currently used may be too high for families with weak histories. If this is true, then it is likely that the risk estimates of family history calculated here are slight overestimates of the true risk. Additional caveats for this work include the fact that, in these data, there is no information on male relatives as well as the fact that previous analyses of these data have indicated that the rates of breast and ovarian cancers are underreported in second-degree relatives. Both of these caveats may have led to underestimation of BRCA1 and BRCA2 mutation carrier probabilities for these women and hence to an overestimation of the remaining effect due to family history.

A final cautionary note must be added. Although the women in this analysis were defined as carriers and noncarriers on the basis of a generalized statistical model, these assignments may not hold true at the individual level. Women with low to moderate risk based on family history and ethnic background may still test positive for BRCA1 and BRCA2 mutations (40). The final determination of carrier status and the remaining role of family history will thus be a continually changing process as the collection of laboratory data proceeds.

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NOTES

¹*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

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Effect of Soy milk Consumption on Serum Estrogen Concentrations in Premenopausal Japanese Women

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Background: Estrogens have been implicated in the development of breast cancer. Preliminary evidence suggests that consumption of soy products, which contain isoflavones (phytoestrogens), can reduce serum estrogen levels. Our purpose was to determine the effect of soy consumption on serum estrogen levels in premenopausal women by use of a dietary intervention approach. **Methods:** Premenopausal Japanese women were randomly assigned to receive either a soy milk-supplemented diet (n = 31) or a normal (control) diet (n = 29). The women in the soy milk-supplemented group were asked to consume about 400 mL of soy milk (containing about 109 mg of isoflavones) daily during a study period that involved three consecutive menstrual cycles. Follicular-phase blood samples were to be obtained in the menstrual cycles preceding (cycle 1) and following (cycle 3) the 2-month dietary intervention. All statistical tests were two-sided. **Results:** At the end of the study period, estrone and estradiol levels were decreased by 23% and 27%, respectively, in the soy milk-supplemented group and were increased by 0.6% and 4%, respectively, in the control group. The changes for each hormone between the two groups were not statistically significantly different. In the soy milk-supplemented group, menstrual cycle length was increased by nearly 2 days, and, in the control group, it was decreased by approximately 1 day, a difference that was not statistically significant. A subgroup analysis restricted to subjects who provided follicular-phase blood samples on the same day or 1 day apart in menstrual cycles 1 and 3 showed a reduction in serum estrone levels in the soy milk-supplemented group that was of borderline statistical

significance ($P = .07$ for change in serum estrone level in soy milk-supplemented group versus control group). **Conclusion:** Much larger studies will be required to confirm the ability of soy products to reduce serum estrogen levels. [J Natl Cancer Inst 1998;90:1830-5]

It has been proposed that intake of isoflavones, i.e., genistein and daidzein (also termed phytoestrogens), which are abundant in soy products, can reduce women's risk of breast cancer (1,2). These phytoestrogens normally compete with estradiol for binding to estrogen receptors (3,4) and may therefore interfere with estrogen-induced cellular proliferation. Studies (5,6) have shown that isoflavones also affect cells by inhibiting their response to growth factors and activation of tyrosine kinase. There are epidemiologic studies that support the hypothesis that soy consumption is associated with lowering of risk of breast cancer (7-12). Nomura et al. (7) found an inverse association between intake of miso soup and subsequent risk of breast cancer. In case-control studies, a significant inverse association between soy consumption and breast cancer risk was observed in Chinese women by Lee et al. (8) but not by Yuan et al. (9). Inverse association between tofu intake and breast cancer risk was reported in Japanese (10) and Asian-American (11) women. A recent study by Ingram et al. (12) demonstrated a reduction of breast cancer risk among women that was associated with high intake of phytoestrogen (as measured by urinary excretion of isoflavones and lignans).

There is a general agreement that hormones, in particular estrogens, are involved in the development of breast cancer (13). Our particular interest has been to study the possibility that soy consumption decreases the serum levels of female steroid hormones. This decrease, in turn, may ultimately help prevent the development of breast cancer. In our recent cross-sectional study (14), we found a negative association between serum estradiol concentration and intake of soy products among premenopausal Japanese women. On the basis of this observation, we have undertaken a dietary intervention trial to assess the influence of soy consumption on the hormonal status of premenopausal Japanese women.

MATERIALS AND METHODS

Subjects and Dietary and Serum Measurements

All female students and teachers (n = 72) (who were premenopausal and not pregnant) at a course given at a nurses' training school in Gifu, Japan, were invited to participate in the present study. Sixty-five of 72 agreed to participate as of April 1997. Of these, three who reported a history of endocrine diseases (diabetes [n = 2] and adrenal disease [n = 1]) and two who were taking hormonal medication were excluded from the study. No one had cancer, chronic hepatitis, or cardiovascular disease. This study was approved by the local institutional review board, and all of the participants provided written informed consent. The remaining 60 women were randomly assigned to either the soy milk-supplemented group or the control group. Women in the soy milk-supplemented group were instructed to consume about 400 mL of soy milk daily that was supplied to them by the study during the dietary study period. Study subjects did not consume soy milk from any source other than what was provided to them. We could not obtain urine samples from the study participants that could have helped to assess their compliance. Soy milk used for this study was purchased from Kibun Food, Chemifa Tokyo, Japan. Isoflavone concentration of soy milk was determined by the Japan Food Research Laboratory, Tokyo, by use of a previously described high-performance liquid chromatographic technique (15). One hundred grams of soy milk (equal to 98.0 mL) contains 0.7 mg of daidzein, 9.4 mg of daidzin, 0.7 mg of genistein, and 16 mg of genistin. The structures of these compounds as well as those of estrone and estradiol are shown in Fig. 1. Women in the control group continued with their usual diet. Both groups were asked to continue their usual lifestyle.

We assumed that the relationship between serum estradiol and isoflavone intake in our previous cross-sectional study was applicable for the prediction of changes in serum estradiol after 2 months of dietary intervention in the present study. We expected a 38% decrease in estradiol concentration by isoflavone intake available from 400 mL of soy milk. We determined that at least 28 subjects in each group were required to have a power of 80% to detect this difference in serum estradiol levels with type I error (α) = 0.05.

Each woman completed a self-administered questionnaire providing basic demographic information and menstrual and reproductive histories before the initiation of the dietary study period.

The first day of the menstrual bleeding was recorded for each woman (day 1), and the dietary study period started on day 11 of the first menstrual cycle (cycle 1). Women in the soy milk-supple-

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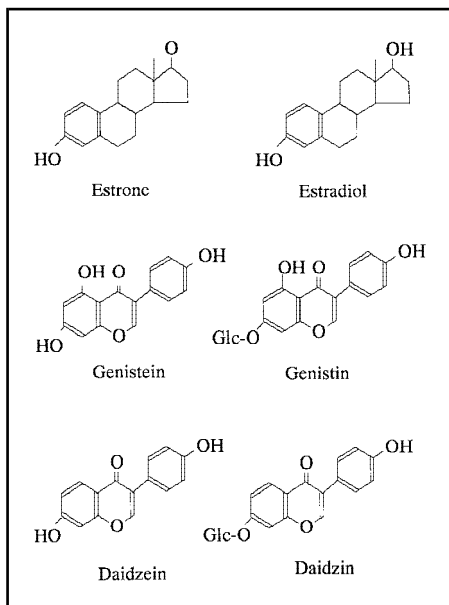


Fig. 1. Chemical structures of estrone, estradiol, genistein, genistin, daidzein, and daidzin.

mented group were instructed to consume about 400 mL of soymilk daily until day 11 of cycle 3. A fasting blood sample was collected on the morning of day 11 of cycle 1 and on day 11 of cycle 3. Each woman completed a series of daily 24-hour diet records from day 2 through day 10 of cycle 1 (diet record 1) and day 2 through day 10 of cycle 3 (diet record 2). In addition, women in the soymilk-supplemented group recorded their soymilk consumption throughout the dietary study period. The intake of all soy products (soymilk, tofu, miso, soybeans, etc.) and the intake of macronutrients and micronutrients were estimated from the diet records using the Standard Tables of Food Composition in Japan, 4th revised edition (16). We approximated isoflavone intake from soy products other than soymilk with the use of data from previous studies (17,18). Detailed information about estimation of isoflavone intake has been described elsewhere (14).

Each woman was weighed before and after the dietary study period. After the dietary study period, the onset dates of the following two menstruations were reported by the subjects.

The blood samples were centrifuged at 1300g for 10 minutes at room temperature within 3 hours of sample collection, and the serum was separated. The samples were divided into 1-mL aliquots and stored at -80°C until assayed. Serum concentrations of estrone, estradiol, and sex hormone-binding globulin (SHBG) were determined by radioimmunoassay using kits purchased from Eiken Chemical Co. Ltd. (Tokyo), Diagnostic Products Cooperation, Japan (Chiba), and Pharmacia & Upjohn Co. Ltd. (Tokyo), respectively. The intra-assay coefficients of variation were 7.4% for estrone, 2.5% for estradiol, and 7.8% for SHBG.

Statistical Analysis

To evaluate the effects of soymilk consumption on hormone status, the concentrations of estrone, estradiol, and SHBG prior to and after the dietary study period were compared with the soymilk-supplemented and control groups using the Mann-

Whitney test. The Mann-Whitney and the Wilcoxon matched pairs signed rank tests were used to compare values of variables at baseline and changes in those variables over the study period. Values for serum hormone concentrations and nutrient intakes were log transformed for Wilcoxon matched pairs signed rank test. Analysis of variance was applied to compare the lengths of the four cycles in each group. All *P* values were calculated from two-tailed tests of statistical significance.

Some blood samples could not be collected on day 11 of cycle 1 or day 11 of cycle 3 in some participants because of school holidays. Actual sampling dates varied from day 9 through day 13 for cycle 1 and from day 7 through day 14 for cycle 3. Therefore, we performed a subgroup ($n = 44$) analysis restricted to subjects who provided blood samples no more than 1 day apart from menstrual cycles 1 and 3.

RESULTS

Sixty women (31 in the soymilk-supplemented group and 29 in the control group) began the study. An initial comparison of preintervention age, height, weight, and other lifestyle variables, such as smoking status, parity, and age at menarche, showed no statistically significant differences between the soymilk-supplemented group and the control group (Table 1).

Prior to random assignment, the initial diet for each group was almost identical with respect to intake of macronutrients and micronutrients as well as soy products and isoflavone (Table 1).

Both the soymilk-supplemented group and the control group significantly decreased their intake of energy and of most nutrients over the dietary study period. In the soymilk-supplemented group, a statistically significant decrease (shown as %) was observed for energy (8.0%), carbohydrate (11.6%), calcium (10.3%), cholesterol (22.0%), carotene (35.7%), vitamins B₂ (15.0%) and C (34.4%), salt (19.4%), and alcohol (43.8%). In the control group, a statistically significant decrease (shown as %) was observed for energy (10.8%), protein (11.1%), fat (12.4%), cholesterol (16.6%), carbohydrate (9.4%), calcium (8.2%), retinol (12.7%), and vitamins B₂ (16.1%) and C (15.6%). However, nutrient densities (calculated as nutrient intake divided by energy) did not change statistically significantly except for increases in protein (9.8%), crude fiber (11.0%), iron (42.6%), and vitamins B₁ (11.8%) and E (23.5%) and decreases in carbohydrate (3.6%), cholesterol (16.6%), vitamin C (31.0%), and salt (11.8%) in the soymilk-supplemented group (data not

shown). In the control group, nutrient densities did not change significantly for any nutrient we tested.

The mean (standard deviation [SD]) daily soymilk consumption estimated from the diet records was 354.8 mL (70.1 mL) in the soymilk-supplemented group. Based on the basis of the records of daily soymilk consumption throughout the dietary study period, the mean (SD) soymilk consumption was 365.0 mL (46.1 mL). The mean isoflavone intake was about 4.5-fold higher at the end of the dietary study period compared with the intake before it. This change was statistically significant ($P = .0001$). Intake of soy products other than soymilk and isoflavone from these products was decreased in terms of the nutrient densities (20.4% and 19.3%, respectively) at the end of the dietary study period in the soymilk-supplemented group, but these differences did not attain statistical significance.

There were no statistically significant changes in isoflavone intake divided by energy in the control group before and after the dietary study period.

The initial and final concentrations of serum hormones are shown for each group in Table 2. The estrone concentration was too low to be measured (<10 pg/mL) in two women in the soymilk-supplemented group during the dietary study period; for analysis purposes, we assigned them a serum estrone concentration of 10 pg/mL. The mean estrone concentration significantly decreased by 23% ($P = .02$) in the soymilk-supplemented group and, in the control group, it increased by 0.6%. The mean estradiol concentrations decreased by 27% in the soymilk-supplemented group and increased by 4% in the control group. However, these changes between the two dietary groups was not statistically significant ($P = .20$ for estrone and $P = .22$ for estradiol). SHBG remained relatively stable in both groups.

The 3rd and 4th menstrual cycles were, on average, nearly 2 days longer than the 1st menstrual cycle in the soymilk-supplemented group, whereas, in the control group, these two cycles were nearly 1 day shorter than the 1st menstrual cycle (Table 3). However, these changes in cycle length were not statistically significant in both groups. There was no signifi-

Table 1. Demographic characteristics and food and nutrient consumption prior to and following the dietary study period

<i>Characteristics</i>						
	Soymilk-supplemented (n = 31)	Control (n = 29)	<i>P</i> *			
Mean (standard deviation)						
Age, y	26.1 (7.9)	26.9 (6.8)	.69			
Weight, kg	50.6 (6.3)	50.7 (5.6)	.91			
Height, cm	157.0 (6.1)	158.7 (4.6)	.23			
Body mass index, kg/m ²	20.6 (2.2)	20.1 (2.0)	.30			
Age at menarche, y	12.4 (1.3)	12.7 (1.1)	.38			
No. (%)						
Parous	4 (12.9)	3 (10.3)	1.00			
Current smoker	9 (29.0)	7 (24.1)	.77			
<i>Nutrients</i> †						
	Soymilk-supplemented (n = 31)			Control (n = 29)		
	Initial	Final	<i>P</i> ‡	Initial	Final	<i>P</i>
Energy, kcal	1706 (256)	1570 (261)	.0001	1665 (191)	1486 (211)	.0001
Protein, g	59.5 (11.2)	60.0 (11.0)	.92	57.8 (7.3)	51.4 (9.7)	.0001
Fat, g	56.8 (9.6)	54.2 (11.7)	.10	55.7 (9.3)	48.8 (12.3)	.002
Cholesterol, mg	296 (92)	231 (85)	.001	265 (60)	221 (80)	.001
Carbohydrate, g	225 (41)	199 (35)	.0001	223 (29)	202 (27)	.001
Crude fiber, g	2.7 (0.7)	2.7 (0.6)	.37	2.6 (0.6)	2.6 (0.9)	.06
Retinol, mg	252 (132)	303 (453)	.59	268 (166)	234 (227)	.04
Carotene, mg	1834 (1207)	1179 (762)	.01	1474 (625)	1644 (959)	.07
Vitamin A, IU	1900 (802)	1719 (1553)	.07	1755 (838)	1736 (973)	.22
Vitamin B ₁ , mg	0.78 (0.14)	0.81 (0.18)	.67	0.78 (0.16)	0.75 (0.14)	.06
Vitamin B ₂ , mg	1.13 (0.24)	0.96 (0.23)	.001	1.18 (0.38)	0.99 (0.25)	.0001
Vitamin C, mg	73.9 (31.0)	48.5 (30.8)	.0001	78.9 (43.4)	66.6 (30.8)	.01
Vitamin E, mg	6.4 (1.5)	7.2 (1.5)	.0001	5.9 (1.2)	5.7 (1.4)	.06
Calcium, mg	437.5 (119.7)	392.5 (97.4)	.04	437.4 (137.0)	401.6 (152.6)	.005
Iron, mg	8.2 (1.7)	10.8 (2.0)	.0001	7.9 (1.5)	7.2 (1.9)	.0007
Salt, g	9.3 (2.1)	7.5 (1.6)	.0001	9.0 (1.5)	8.7 (1.6)	.24
Alcohol, mL	6.3 (10.4)	3.6 (7.7)	.001	3.5 (4.9)	2.7 (4.6)	.30
<i>Soy products</i> †						
	Soymilk-supplemented (n = 31)			Control (n = 29)		
	Initial	Final	<i>P</i>	Initial	Final	<i>P</i>
Soymilk, g	0.6 (3.6)	361.9 (71.5)	.0001	0.8 (3.9)	0.0 (0.0)	1.00
Isoflavone from soymilk, mg	0.2 (0.9)	97.0 (19.2)	.0001	0.2 (1.0)	0.0 (0.0)	1.00
Other soy products, g						
Miso	6.4 (5.3)	5.0 (3.8)	.11	5.3 (3.8)	4.5 (4.1)	.35
Tofu	26.8 (44.8)	23.5 (25.7)	.58	22.0 (21.0)	21.4 (22.4)	.52
Dry tofu	0.01 (0.06)	0.004 (0.02)	.75	0.01 (0.04)	0.01 (0.04)	.53
Fried tofu	3.6 (3.5)	1.2 (1.7)	.0004	2.7 (4.7)	1.6 (2.9)	.29
Boiled soybeans	4.3 (11.6)	3.7 (9.9)	.35	1.5 (2.0)	1.4 (2.8)	.50
Dry soybeans	1.2 (3.4)	0.7 (1.8)	.42	0.4 (1.1)	0.2 (0.8)	.07
Fermented soybeans	3.2 (5.9)	1.8 (3.3)	.30	4.5 (5.8)	3.9 (6.5)	.79
Yuba	0.0 (0.0)	0.0 (0.0)	—	0.02 (0.05)	0.0 (0.0)	.25
Soy sauce	14.3 (7.6)	9.4 (4.7)	.0001	12.2 (5.0)	10.7 (6.0)	.09
Total	60.0 (56.7)	45.4 (36.0)	.02	48.8 (27.3)	43.9 (27.5)	.07
Isoflavone from other soy products, mg	25.2 (23.4)	19.4 (15.0)	.04	20.7 (12.9)	18.4 (13.4)	.046

*Two-sided *P* values were calculated for difference in means using Student's *t* test. Parity and smoking were evaluated using the Fisher's exact test.

†Mean (standard deviation).

‡Two-sided *P* values were calculated using the Wilcoxon matched pairs signed rank test.

cant difference in the mean length of the four menstrual cycles between the two groups (the means [SD] were 31.1 [4.3] and 30.3 [2.9] days in the soymilk-supplemented and the control groups, respectively).

We restricted our statistical analysis to women who provided blood samples no

more than 1 day apart in cycles 1 and 3 (21 women in the soymilk-supplemented group and 23 women in the control group). Among these women, the days of blood collection ranged from day 9 through day 12 in cycle 1 as well as in cycle 3. There were no significant differences in the hormone concentrations at

baseline between the two groups. The mean (SD) of soymilk intake per day was 360.7 mL (68.4 mL) in the soymilk-supplemented group. The mean (SD) of estimated isoflavone intake collectively from soymilk and other soy products was 29.5 mg (27.1 mg) and 121.2 mg (22.3 mg) at baseline and at the end of the di-

Table 2. Serum estrogen and sex hormone-binding globulin (SHBG) concentrations prior to and following the dietary study period

<i>Entire group</i>							
	Soymilk-supplemented (n = 31)			Control (n = 29)			<i>P</i> §
	Initial*	Final*	<i>P</i> ‡	Initial*	Final*	<i>P</i> ‡	
Estrone, pg/mL	41.1 (30.2)	31.6 (25.7)	.02	41.1 (25.4)	41.4 (30.9)	.73	0.20
Estradiol, pg/mL	87.5 (78.9)	63.6 (50.3)	.12	86.8 (71.7)	90.2 (84.7)	.70	0.22
SHBG, nmol/L	60.9 (20.7)	60.5 (20.6)	.95	65.1 (23.2)	67.4 (25.9)	.36	0.65

<i>Selected group†</i>							
	Soymilk-supplemented (n = 21)			Control (n = 23)			<i>P</i> §
	Initial*	Final*	<i>P</i> ‡	Initial*	Final*	<i>P</i> ‡	
Estrone, pg/mL	45.5 (32.7)	31.8 (29.4)	.005	42.8 (28.3)	44.1 (32.2)	.81	0.07
Estradiol, pg/mL	98.0 (85.0)	65.4 (51.7)	.10	84.8 (74.8)	93.6 (90.1)	.60	0.13
SHBG, nmol/L	64.9 (22.9)	62.7 (23.2)	.49	64.7 (25.8)	66.0 (28.8)	.76	0.50

*Mean (standard deviation).

†Women from whom blood samples were obtained on the same day or 1 day apart of the menstrual cycles 1 and 3.

‡Two-sided *P* values were calculated using the Wilcoxon matched-pairs sign rank test.§Two-sided *P* values were calculated for the differences in changes between the two groups by use of the Mann-Whitney test.**Table 3.** Menstrual cycle length (days)* for each group during and after the dietary study period†

<i>Entire group</i>		
Cycle	Soymilk-supplemented (n = 31)	Control (n = 29)
1st	30.1 (4.6)	31.7 (6.4)
2nd	30.7 (5.6)	30.7 (5.4)
3rd	32.1 (6.5)	31.0 (8.2)
4th	31.7 (7.5)	29.8 (3.6)
	F = 1.00 (<i>P</i> = .40)§	F = .27 (<i>P</i> = .84)§

<i>Selected group‡</i>		
Cycle	Soymilk-supplemented (n = 21)	Control (n = 23)
1st	29.0 (4.2)	31.6 (6.9)
2nd	29.9 (4.9)	30.4 (5.5)
3rd	31.1 (6.5)	31.7 (9.1)
4th	32.4 (8.7)	29.0 (3.1)
	F = 1.83 (<i>P</i> = .15)§	F = 0.47 (<i>P</i> = .70)§

*Mean (standard deviation).

†From day 11 of the 1st menstrual cycle to day 10 of the 3rd menstrual cycle.

‡Women from whom blood samples were obtained on the same day or 1 day apart of the menstrual cycles 1 and 3.

§All *P* values are two-sided.

etary study period, respectively, in the soymilk-supplemented group. The corresponding figures for isoflavone intake in the control group were 22.0 mg (12.7 mg) and 20.6 mg (14.0 mg), respectively. Changes in intake of nutrients as well as isoflavone in the selected subgroups were similar to those observed for the complete subgroups, i.e., in all subjects (data not shown). Serum estrone concentration was significantly decreased by 30.1% (*P* = .005) in the soymilk-supplemented group when the comparison was between values before and after the dietary study period and increased by 3% in the control group,

respectively, although the difference in the change between the two groups was of only borderline significance (*P* = .07) (Table 2). Estradiol concentrations decreased by 33.2% in the soymilk-supplemented group and increased by 10% in the control group, respectively, but these changes in values were not significantly different when both groups were compared. The 4th menstrual cycle was 3.4 days longer than the 1st menstrual cycle in the soymilk-supplemented group and 2.6 days shorter in the control group (Table 3). However, these changes did not attain statistical significance.

DISCUSSION

Our results support the hypothesis that soy consumption alters circulating ovarian steroid hormone concentrations in premenopausal women. To our knowledge, only three studies have previously evaluated the effect of soy diet on the estrogen status of premenopausal women (19–21). However, all of these studies were small (fewer than 15 subjects) and did not include a control group. In our study, we randomly allocated subjects to an experimental or a control group. The sample size of our study was larger than that in the previous studies, although it was not large enough to obtain sufficient power. With this sample size, the power of finding a significant difference in estrone concentration between the two groups was only 53%.

We should remark that, unlike published dietary intervention studies employing soy foods, in our study, subjects in both the soymilk-supplemented group and the control group consumed soy products at baseline. We thus essentially investigated the effect of higher versus lower level of soy intake on hormone concentrations.

Lu et al. (20) found decreased estradiol concentrations in six healthy premenopausal women during soy feeding of three 12-oz portions per day of soymilk (about 200 mg of isoflavones per day) for 1 month. Cassidy et al. (19) observed that the midcycle peaks of luteinizing hormone and follicle-stimulating hormone

were suppressed, but estradiol was increased during dietary intervention with soy protein (60 g soy protein containing 45 mg conjugated isoflavones). They later reported no changes in estradiol, luteinizing hormone, and follicle-stimulating hormone during the diet with a half dose of conjugated isoflavones or the same dose of unconjugated isoflavones (22). Petrakis et al. (21) reported an increase in estradiol during the 6 months of soy consumption (38 g of soy protein isolate containing 38 mg of genistein). In their study, blood measurements were not taken at the same time point in the menstrual cycle. Therefore, changes in estradiol concentration were estimated using computer-generated best-fit curves.

The findings regarding changes in serum estradiol levels in previous studies are somewhat contradictory, which may be due to variations in the quantity of isoflavones consumed. Petrakis et al. (21) postulated that the sporadically elevated estradiol concentration observed in their study during the soy diet may represent evidence of competition between binding of estradiol and isoflavones to the estrogen receptors. Although serum estradiol levels may be increased by relatively low isoflavone intake as observed in the studies by Cassidy et al. (19) and Petrakis et al. (21), high intake of isoflavones, due to the estrogenicity, may cause a decrease in the serum levels of luteinizing hormone and follicle-stimulating hormone. This may lead to a decrease in serum estrogen concentrations. In the present study and the study reported by Lu et al. (20), the experimental subjects received a relatively large amount of isoflavones (about 100 and 200 mg, respectively), and both studies observed about a 30% decrease in serum estradiol concentration in the follicular phase. It is possible that isoflavones alter estradiol concentration through alteration of estrogen metabolism. The *in vitro* findings have shown that genistein antagonizes transforming growth factor- α -induced synthesis of estrogen in granulosa and theca cells (23) and inhibits the activity of 17 β -hydroxysteroid oxidoreductase type I, an enzyme that converts estrone to estradiol (24). The interaction between isoflavones (or their metabolites) and intestinal steroid hormone metabolism may be related to estradiol reduction. A similar type of interaction is postulated for the relation-

ship between fiber intake and estradiol (25).

No data have been previously published on the effects of soy consumption on serum estrone levels. A trend toward decrease in serum estrone seen in the present study provides additional support for the hypothesis that isoflavones may have the ability to reduce the synthesis of estrogens.

There was a suggestion of an increase in menstrual cycle length after the soy diet, although this did not attain statistical significance. Prolongation of menstrual cycle length after dietary intervention has been reported by Cassidy et al. (19) and Lu et al. (20).

The mean cycle length of the 1st menstrual cycle was 2.6 days longer than that of the 4th menstrual cycle in the control group in the selected subgroup analysis (Table 3). The reason why the length of the menstrual cycle of control groups decreased over the study period is unclear. It is possible that some factors other than diet may have been affecting cycle length and, therefore, the observed difference in cycle length changes between the two groups may be overestimated.

Serum estrone and estradiol levels fluctuate during the menstrual cycle. Estrogen concentrations in the soymilk-supplemented group may appear to be lower than those in the control group because increases in cycle length most likely reflect the elongation of the follicular phase (26). Data on hormone concentrations of study participants throughout the menstrual cycles were unavailable. Slight increases of estrone and estradiol levels in the control group may have been related to the decreased cycle length. However, despite changes in cycle length, estrone and estradiol concentrations were quite stable in the control group compared with the soymilk-supplemented group. Since the fluctuation in levels of serum estrogens is part of natural physiology, it is important to emphasize that effects of soy supplementation may not be visible when the study size is not large. A much larger study (by size and duration) and more frequent determinations of serum estrogen levels are required to offset the variations due to natural physiologic changes.

We did not completely control the diet by providing the subjects with all foods during the study period. The diet was assessed using self-reported diet records.

However, biased reporting by any one study group is unlikely, and the comparison of dietary changes between the two groups is considered valid. Both dietary groups showed a decrease in energy intake and a decrease in consumption of some macronutrients based on the diet records. It could be due to seasonal change or omission of some foods they had as they became tired of keeping records. These reasons are unlikely to be dependent on the dietary group. In terms of nutrient densities, the change was mainly the intake of nutrients rich in soymilk in the soymilk-supplemented group. Relatively less control of diet and lifestyle during the study period likely enhanced participation rates, strengthening the generalizability of these findings.

The present study suggests that high intake of soymilk may modify circulating estrogen concentrations and possibly alter menstrual cycle length, both of which may be potentially beneficial for lowering the risk of breast cancer. Much larger studies are required to confirm the ability of soy products to reduce serum estrogen levels.

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NOTES

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