AD NUMBER

ADB240578

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info; Sep 98. Other requests shall be referred to USAMRMC, Ft Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, 1 Jun 2001.

AD_____

AWARD NUMBER DAMD17-96-1-6284

TITLE: Ethnicity, Soybean Consumption, and Mammographic Densities

PRINCIPAL INVESTIGATOR: Gertraud Maskarinec, Ph.D., M.D.

CONTRACTING ORGANIZATION: University of Hawaii
Honolulu, Hawaii 96822

REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1998	Annual (1 Sep 97 - 31 Aug 98)

4. TITLE AND SUBTITLE
Ethnicity, Soybean Consumption, and Mammographic Densities

5. FUNDING NUMBERS
DAMD17-96-1-6284

6. AUTHOR(S)
Gertraud Maskarinec, Ph.D., M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Hawaii
Honolulu, Hawaii 96822

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, September 1998). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The purpose of this study is to determine whether mammographic density patterns differ by ethnic background and to explore the possible association of a soy rich diet with mammographic density patterns. So far, 693 women have been recruited at mammography clinics in Honolulu and 465 women have completed all questionnaires. Mammographic density assessment and an exploratory analysis has been performed for 458 women for whom mammograms were available. Preliminary results on mammographic density suggest that the area of dense tissue in the breast may be smaller in Asian than in Caucasian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Asian women may be equal to or higher than in Caucasian women. The strongest determinants of mammographic density patterns appear to be body mass index, age, and estrogen replacement therapy. We have identified associations of several reproductive and dietary factors with mammographic densities. Whereas the relation between tofu and mammographic densities was inverse before menopause, the relation was direct after menopause. Quality control of mammographic density assessments and more detailed statistical analyses are necessary to complete this project.

14. SUBJECT TERMS
Breast Cancer
Nutrition, Soy, Mammographic Densities

15. NUMBER OF PAGES
29

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

__N/A__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Leonard Marchand 9-21-98

PI - Signature Date

Table of Contents

Introduction

A possible association between mammographic parenchymal patterns and breast cancer risk has been shown in numerous studies[1-4]. Parenchymal patterns refer to the distribution of fat and connective and epithelial tissue in the female breast. Fat appears dark on mammograms and the other tissues appear light. Since fat tissue is not at risk to become cancerous, the radiographically light areas, the mammographic densities, are thought to be relevant to breast cancer risk. Monitoring changes in mammographic density might offer a non-invasive method to evaluate preventive strategies and to select populations at high cancer risk for interventions. A recent publication from Canada[5] reported a decrease in mammographic density patterns as a result of a low-fat, high-carbohydrate dietary intervention.

Based on the observation that breast cancer incidence rates differ among ethnic groups in Hawaii, our study hypothesis is that mammographic density patterns vary among women from different ethnic groups. In 1986-1990, the breast cancer incidence rate (age-adjusted to 1970 U.S. population) for Caucasians was $133/10^5$, for Hawaiian/Part Hawaiians $113/10^5$, for Japanese $89/10^5$, for Filipina $57/10^5$, and for Chinese $58/10^5$ (Hawaii Tumor Registry, unpublished report). Therefore, it was proposed that women from ethnic groups with high breast cancer risk are more likely to have a dense parenchymal pattern than women from ethnic groups at low risk for breast cancer. Intake of soy products was suggested as one of the dietary factors that may protect Asian women from breast cancer[6].

The purpose of this study is to determine whether mammographic density patterns differ by ethnic background and to explore the possible association of a soy rich diet with mammographic density patterns. Asian populations have traditionally consumed large amounts of soybeans, a major source of phytoestrogens, in particular isoflavones, heterocyclic phenols similar in structure to natural and synthetic estrogens[6,7]. The hypothesis that these weakly estrogenic substances may exhibit anti-estrogenic properties by competing for estrogen receptors and protect women against breast cancer has been supported by animal studies[7,8] demonstrating anti-estrogenic and antineoplastic effects.

The specific objectives of this project are to determine whether mammographic density patterns differ among women of Japanese, Filipino, Hawaiian, and Caucasian ancestry after adjusting for age, family history of breast cancer, reproductive and anthropometric factors, to explore the possible association of a soy rich diet on mammographic parenchymal patterns while controlling for confounding variables, and to investigate the association between urinary isoflavone levels and self-reported soy intake. The last specific aim has been accomplished and the results have been published recently[9] (see appendix). Several abstracts using preliminary data have also been presented at professional meetings (see appendix). This second annual report concentrates on actions and analyses that were achieved during the second year of the study.

Methods

Recruitment and data collection: During the first year, women were recruited at three mammography facilities in four locations on the island of Oahu: Kapiolani Women's Center, Kaiser Permanente Honolulu and Punawai Clinics, and St. Francis Hospital. In the second year we added Waianae Comprehensive Health Center, a rural clinic covering primarily Native Hawaiians because we wanted to increase the number of Native Hawaiian women enrolled in this study. All study participants signed informed consent and completed a questionnaire asking for a detailed dietary and reproductive history, as well as a soy food questionnaire. Mammography films were obtained from the mammography clinics after the radiologic evaluation had been completed and ruled out any malignancy. A subset of 102 women donated an overnight urine sample. Details of the recruitment procedures, the data collection, and the urinary analysis were described in the first annual report.

Mammogram density assessment: Mammograms were requested in batches from the clinics after the radiologists had completed their evaluation. The two cranio-caudal mammogram films were scanned into a PC. During the fall of 1997, we installed an improved X-ray digitizer (Cobrascan CX-612-T) that provides better quality images. Computerized mammographic density assessment was performed using a method that was first developed in Toronto[10] and later modified at the University of Southern California in Los Angeles[11]. The reader first draws the outline of the breast (using an outlining tool) and then searches for the best threshold gray level value X where all pixels with values above X are considered to represent mammographic densities. The pixel count corresponding to the area colored within the outline of the breast is determined by the computer, as is the total area within the outline of the breast. The proportion of the breast with densities is calculated as the ratio of the colored area to the total area of the breast. So far, two readers have performed mammographic density assessment for all mammograms. We are planning to have Dr. Giske Ursin from the University of Southern California assess a sample of mammograms for quality control and to help resolve divergent readings.

Statistical analysis: We inspected the data for missing values and errors and performed the appropriate corrections or replacements. Several variables showed non-normal distributions and were transformed using their natural logarithms. If necessary, we created categorical variables. Student's t-tests and χ^2 tests[12] were applied to assess differences between groups. Pearson's correlation coefficient and multiple linear regression models were used to explore relations between variables. All analyses were performed using PC-SAS®, release 6.12 (SAS Institute, Cary, NC).

Preliminary Results

By August 1998, 693 women had indicated their interest in our study by completing one of the recruitment flyers. Of these women, 371 were recruited at Kapiolani Women's

Center, 269 at the Kaiser Honolulu Clinic, 13 at the Kaiser Punawai Clinic, 7 at St. Francis Hospital, and 33 at the Waianae Coast Comprehensive Health Center. Overall, 465 women signed informed consent and returned the questionnaires giving a 67% response rate. The return rates differed by clinic: 78% at Kapiolani Women's Center, 55% at the Kaiser Honolulu Clinic, 54% at the Kaiser Punawai Clinic, 29% at St. Francis Hospital, and 61% at the Waianae Coast Comprehensive Health Center. Seven of the 465 women had to be excluded from the analysis either because their mammograms could not be found or because they had breast implants. The median age of the 458 women who returned the questionnaire and had a mammogram available was 53 years (range: 35 to 85 years). The ethnic distribution was as follows: 166 (36.2%) Caucasian, 64 (14%) Chinese, 16 (3.5%) Filipino, 54 (11.8%) Native Hawaiian, 145 (31.7%) Japanese, and 13 (2.8%) others.

So far, mammographic density analysis has been completed for 458 women. Among this group of women, the mean area of the breast was approximately 50% larger for Caucasian and for Native Hawaiian as for Japanese and Chinese women (Table 1). While the mean dense area was 15% smaller in Japanese and Chinese women, the percent densities was considerably higher in Asian women than in Caucasian and Native Hawaiian women. Women who reported current estrogen replacement therapy (Table 2), had larger dense areas and higher percent densities than women who did not report current estrogen use.

Reproductive behavior differed among ethnic groups (Table 3). Women with Chinese and Japanese ancestry were more likely than Caucasian women to experience menarche when they were older than 12 years. The Caucasian group had the smallest proportion of women who reported a live birth before age 30. Native Hawaiian women gave birth to the greatest number of children. Current estrogen replacement therapy varied between a high of 63% among women with Japanese ancestry and a low of 44% among women with Filipino and other ancestries.

Dietary habits (Table 4) were related to ethnic background. The mean daily calorie intake was considerably lower for women with Chinese and Japanese background than for the other groups. In all groups, approximately 30% of calories were fat calories and the mean fruit and vegetable intake was between 4 and 5 servings per day suggesting that the study population had relatively healthy eating habits. Tofu intake was less than twice as high among Caucasian women than in all other groups.

Of all variables tested so far, body mass index (BMI) had the highest correlation with mammographic densities (Table 5). This was true for pre- and postmenopausal women. In addition, daily caloric intake and tofu intake were significantly related to mammographic densities among premenopausal women, while only tofu intake was significant among postmenopausal women. Whereas the relation between tofu and mammographic densities was inverse before menopause, the relation was direct after menopause. Stratification by estrogen use did not change the association of BMI and tofu with mammographic densities (Table 6). However, the relation between fat intake

and mammographic densities became borderline significant among estrogen users, while it remained close to zero among non-users. In an exploratory multiple linear regression (Table 7), 43% of the variation in percent densities could be explained. Body mass index and age were the strongest contributors to the model, whereas Caucasian ethnicity, estrogen use, menopausal status, and age at first live birth contributed small amounts of variance to the model.

Discussion

Overall recruitment for this study is nearly complete. We are considering to enroll additional women from the Hawaii Department of Health's Breast and Cervical Cancer Screening Program. This federally funded program provides mammography services predominantly to low-income women, many of whom are of Filipino and Native Hawaiian ethnicity. Recruitment among these ethnic groups has been a problem, attendance in mammography clinics are low, and the response rates have been poor.

We have made progress in mammographic density assessment and performed initial readings. However, we need to work on the quality control aspects of the density assessment and reconcile readings that strongly disagreed between the two readers.

The preliminary data on mammographic density suggest that the area of dense tissue in the breast may be smaller in Japanese and Chinese than in Caucasian and Native Hawaiian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Chinese and Japanese women may be equal to or higher than in Caucasian and Native Hawaiian women. It appears that the differences between the ethnic groups are not as great as anticipated, possibly due to selection bias. Although breast cancer incidence rates still differ among ethnic groups in Hawaii, the differences have become smaller. Assuming that Asian women with a higher breast cancer risk are more likely to participate in mammography screening and in a research study, the Japanese and Chinese women in this study may have a breast cancer risk that is fairly close the risk experienced by Caucasian women. We plan to explore this issues by using published breast cancer risk estimation models[13]. Because only a small number of recent immigrants whose breast cancer risk is still close to that of their country of origin have participated in this study, the observed differences in mammographic densities may be relatively small in Hawaii.

We have only started to analyze the dietary information collected with the food frequency questionnaires. We are working with a nutritionist to investigate the various dietary variables, to consider adjustment for caloric intake and other important confounders, to select the appropriate regression models, and to explore interactions between variables. It is a challenge to incorporate the numerous reproductive, anthropometric, and dietary variables which are all interrelated into a realistic model. The preliminary observation that the association between tofu and mammographic densities differs by menopausal status warrants further exploration. This finding may be

related to the hypothesis that phytoestrogens contained in soy have an antiestrogenic effect before menopause and an estrogenic effect after menopause.

Conclusions

Preliminary analysis of data collected so far appear to be in support of our hypothesis that women at low risk for breast cancer have fewer mammographic densities. Some associations between mammographic densities and dietary factors have been identified.

References

1. Warner E, Lockwood G, Tritchler D, Boyd NF. The risk of breast cancer associated with mammographic parenchymal patterns: a meta-analysis of the published literature to examine the effect of method of classification. Cancer Detect Prev 1992;16:67-72.
2. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995;87:670-5.
3. Oza AM, Boyd NF. Mammographic parenchymal patterns: a marker of breast cancer risk. Epidemiol Rev 1993;15:196-207.
4. Gail MH, Benichou J. Assessing the risk of breast cancer in individuals. In: DeVita VTJ, Hellman S, Rosenberg SA, eds. Cancer prevention. Philadelphia: JB Lippincott, 1992:1-15.
5. Boyd NF, Greenberg C, Lockwood G, et al. Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. J Natl Cancer Inst 1997;89:488-96.
6. Messina M, Barnes S. The role of soy products in reducing risk of cancer. J Natl Cancer Inst 1991;83:541-6.
7. Messina MJ, Persky V, Setchell KD, Barnes S. Soy intake and cancer risk: A review of the in vitro and in vivo data. Nutr Cancer 1994;21:113-31.
8. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. In: Pariza M, ed. Mutagens and carcinogens in the diet. 1st ed. New York: Wiley-Liss, Inc., 1990:239-53.
9. Maskarinec G, Singh S, Meng L, Franke AA. Dietary soy intake and urinary isoflavone excretion among women from a multi-ethnic population. Cancer Epidemiol, Biomarkers Prev 1998;7:613-9.
10. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629-38.
11. Ursin G, Astrahan MA, Salane M, et al. The detection of changes in mammographic densities. Cancer Epidemiol, Biomarkers Prev 1998;7:43-7.
12. Armitage P. Statistical methods in medical research. Oxford: Blackwell Scientific Publications, 1971.
13. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879-86.

Table 1. Mammographic Densities by Ethnicity

Ethnicity	Caucasian	Chinese	Other	Hawaiian	Japanese
Number	165	64	29	54	145
Age	54	55	50	52	52
BMI	24.9	22.9	25.1	30.5	23.1
Breast size (10^3 pixels)	1234	872	1108	1423	813
Dense areas (10^3 pixels)	302	260	314	319	264
Percent Densities	29%	35%	34%	26%	35%

Table 2. Mammographic Densities by Estrogen Use Postmenopausal Women only

	Estrogen Use	No Estrogen Use
Number	156	112
Age	58	61
BMI	24.7	25.2
Breast size in 10^3 pixels	1087	1215
Dense areas in 10^3 pixels	289	241
Percent Densities	29%	25%

Table 3. Reproductive Variables by Ethnicity

Ethnicity	Caucasian	Chinese	Other	Hawaiian	Japanese
Menarche >12 yrs	46%	30%	41%	43%	39%
AFLB < 30 yrs	49%	61%	62%	74%	60%
No. of children	1.6	2.1	2.2	2.8	1.9
Postmenopause	60%	61%	62%	59%	55%
Estrogen use	60%	54%	44%	56%	63%

Table 4. Dietary Intake by Ethnicity

Ethnicity	Caucasian	Chinese	Other	Hawaiian	Japanese
Tofu (g/day)	9.6	18.6	27.3	23.0	24.4
Fruit servs/day	2.2	2.3	2.2	3.0	2.1
Vegetable s/day	4.4	4.7	5.4	5.1	4.3
Daily calories	2060	1871	2764	2844	2072
Percent fat calories	29.9	26.7	30.6	30.1	29.4

Table 5. Spearman Correlation Coefficients Between Diet and Mammographic Densities

	Premenopausal r_s (p-value)	Postmenopausal r_s (p-value)
Number	190	268
Daily calories	- 0.16 (0.02)	- 0.01 (0.84)
BMI	- 0.66 (0.0001)	- 0.57(0.0001)
Percent fat	- 0.06 (0.43)	- 0.03 (0.66)
Fruits&vegetables (serv.)	- 0.04 (0.56)	- 0.04* (0.56)
Tofu (g/day)	- 0.14* (0.06)	0.19 (0.002)

* Correlation with dense area, all others correlation with percent densities

Table 6. Spearman Correlation Coefficients Between Diet and Mammographic Densities by Estrogen Use

	No Estrogen Use r_s (p-value)	Estrogen Use r_s (p-value)
Number	112	156
Daily calories	0.008 (0.93)	- 0.05 (0.51)
BMI	- 0.67 (0.0001)	- 0.48 (0.0001)
Percent fat calories	0.03 (0.74)	- 0.14* (0.08)
Fruits&vegetables (serv.)	- 0.09* (0.33)	- 0.05 (0.57)
Tofu (g/day)	0.23 (0.01)	0.16 (0.05)

* Correlation with dense area, all others correlation with percent densities

Table 7. Determinants of Percent Densities
Multiple Linear Regression (N=457)

Variable	Parameter Estimate	Partial R^2	Prob>F
BMI (log)	- 1.886	0.30	<0.0001
Age	- 0.015	0.09	<0.0001
Caucasian ethnicity	- 0.242	0.02	<0.0001
Estrogen replacement	0.245	0.01	0.04
Menopause	- 0.232	0.01	0.01
AFLB 30+	0.106	0.004	0.07
Complete model		0.43	0.0001

Dietary Soy Intake and Urinary Isoflavone Excretion among Women from a Multiethnic Population[1]

Gertraud Maskarinec,[2] Seema Singh, Lixin Meng, and Adrian A. Franke

Cancer Research Center of Hawaii, Honolulu, Hawaii 96813

Abstract

Isoflavones are present in soybeans and its products in concentrations up to 300 mg/100 g, have estrogenic and antiestrogenic properties, and may be protective against hormone-related cancers. The purpose of this cross-sectional study was to investigate the association between urinary isoflavone excretion and self-reported soy intake. A total of 102 women of Caucasian, Native Hawaiian, Chinese, Japanese, and Filipino ancestry completed a dietary questionnaire for soy products consumed during the last year and during the 24-h period before urine collection. Overnight urine samples were analyzed for coumestrol and the soy isoflavones genistein, daidzein, and glycitein and their main human metabolites by reverse-phase high-pressure liquid chromatography. Soy protein and isoflavone intake (predominantly from tofu) were estimated using published nutritional databases. Wilcoxon's rank-sum test scores and Spearman rank correlation coefficients were computed.

Japanese women excreted more daidzein, genistein, and glycitein than did Caucasian women, whereas Caucasian women excreted slightly more coumestrol. Soy intake differed significantly among ethnic groups. Dietary soy protein and isoflavone intakes during the previous 24 h were positively related to urinary isoflavone excretion [$r_S = 0.61$ ($P < 0.0001$) and 0.62 ($P < 0.0001$), respectively]. Urinary excretion of isoflavones was also related to annual dietary soy protein and isoflavone intake [$r_S = 0.32$ ($P < 0.0012$) and 0.31 ($P < 0.0016$), respectively]. The strong correlation between urinary isoflavone excretion and self-reported soy intake validates the dietary history questionnaire that is now used in a study exploring dietary risk factors for breast cancer.

Introduction

The low risk for breast and prostate cancer in Asian populations combined with evidence from migration studies suggests that environmental factors such as diet may influence the occurrence of these diseases (1–5). From 1988 to 1992, breast cancer incidence for ethnic groups in the United States (per 100,000 women, age-adjusted to the 1970 United States population) was as follows: Caucasians, 111.8; Native Hawaiians, 105.6; Japanese, 82.3; Chinese, 55; and Filipino, 73.1 (6). These variations have been attributed to differences in dietary fat and fiber intake and possibly to soy consumption among Asian women (7–9). The interest in dietary soy has arisen from findings related to the potential cancer-protective properties of isoflavones (10, 11). Isoflavones are plant products with estrogenic activity and are therefore phytoestrogens, which include a wide variety of phytochemicals such as coumestans and lignans (12, 13). The term phytoestrogen was coined after the observation that Australian sheep grazing on a certain type of subterranean clover had a high rate of infertility (14), an observation later attributed to the high isoflavone content of this plant. The isoflavones have a heterocyclic phenol structure that closely resembles estrogens and enables them to bind to estrogen receptors and to exert weak estrogenic effects (13, 15–17). They may act as antiestrogens by competing with endogenous estrogen for receptor binding, thereby possibly reducing the risk for breast cancer by decreasing the promotional effects of high levels of endogenous estrogens (10, 18, 19) or by altering estrogen biosynthesis (20, 21). Alternative mechanisms suggested for isoflavones to prevent cancer and other chronic conditions are modification of enzyme activity, such as topoisomerase or tyrosine protein kinases (22) that play a role in cell proliferation and transformation, as well as breast cancer oncogene expression (23), inhibition of angiogenesis (24), and apoptosis (25).

Soybeans contain high amounts (concentrations of up to 100–300 mg/100 g) of the glycosides of the isoflavones daidzein, genistein, and glycitein (26–29). Daidzein present in soy foods is partially converted by the gut flora into equol and DMA[3] as end products (30, 31). The other common dietary isoflavone, genistein, is converted to *p*-ethylphenol (31). The uncertain metabolism of soy isoflavones led to the identification of several minor intermediate metabolites (32). The isoflavones and their metabolites can be measured in food, plasma, urine, and feces using gas chromatography and HPLC (27, 33–41).

Studies among various populations and dietary groups have shown large amounts of isoflavonoids in the plasma, urine, and feces of vegetarians; macrobiotics; and in Japanese individuals consuming a traditional Japanese diet (37, 38). Whereas substantial variations among individuals in the excretion of isoflavones and their metabolites have been described (39), information on ethnic differences in soy intake and the excretion of urinary isoflavones is limited (42). Hawaii is an excellent location to explore the possible effects of soy product consumption, due to its multiethnic population with a high

Received 2/17/98; revised 4/13/98; accepted 4/22/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Department of the Army Grant 17-96-1-6284 and NIH Grant 5-K12 CA 01708.

[2] To whom requests for reprints should be addressed, at Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813.

[3] The abbreviations used are: DMA, *O*-desmethylangolensin; HPLC, high-pressure liquid chromatography; i.d., inside diameter.

proportion of women of Asian ancestry who have maintained some of their traditional dietary habits. The objectives of this cross-sectional study were to explore the variation in dietary soy intake and urinary isoflavonoid excretion among women of Japanese, Filipino, Native Hawaiian, Chinese, and Caucasian ancestry and to investigate the association between dietary soy intake and urinary isoflavone excretion.

Materials and Methods

Study Population. Hawaii has a multiethnic population with approximately 24% Caucasian, 20% Japanese, 11% Filipino, 19% Native Hawaiian, 5% Chinese, and 21% other ethnic groups (43). Participating women were recruited from two large mammography clinics in Honolulu. The institutional review boards at all participating organizations approved the study protocols. Women interested in participating received dietary questionnaires and consent forms with addressed return envelopes by mail. In appreciation for their cooperation, women were sent a summary of their dietary intake of various macro- and micronutrients at the end of the study. Mammograms for all participating women were obtained from the clinics after the radiological evaluation was completed. Women with a history of breast cancer, a previous breast biopsy or surgical procedure, or a mammogram with a suspicious lesion requiring biopsy were excluded from the study. Antibiotic use or other metabolic conditions that might affect isoflavone uptake or metabolism were not assessed.

Study participants with mixed ancestry were classified using both parents' ancestry and the following rules. If a person's ethnicity is recorded as a combination of Native Hawaiian and any other ethnicity, the summary code is Native Hawaiian. Otherwise, the summary code is the first stated non-Caucasian ethnicity. Although all Native Hawaiian women were of mixed ancestry, the majority (84%) of Asian women reported one ancestry only.

Dietary Assessment. A self-administered questionnaire was used to obtain demographic and reproductive information and a family history for each participant. The dietary history questionnaire collected the usual frequency of consumption and portion size for more than 50 food items, alcoholic and nonalcoholic beverages, and vitamin supplements during the previous year. A separate questionnaire listing 12 soy-based foods with local names was used to collect information on soy consumption. We used United States Department of Agriculture Food Composition tables (44) to compute soy protein intake and used isoflavone data from food analyses to estimate isoflavone intake (45).

Urine Collection. An overnight urine sample was collected for 102 study participants, and a 24-h dietary questionnaire for soy-based foods was obtained at the same time. Of 129 women eligible for urine collection, 5 women refused to provide a sample, 2 women were menstruating, 8 women were on vacation, 3 women lived too far away, and 9 women could not be reached after repeated calling, resulting in a participation rate of 78%. A container with 0.2 g of ascorbic acid and 0.3 g of boric acid (to prevent bacterial contamination and degradation of analytes) was delivered to each woman. Women were instructed to collect the first urine sample in the morning and all of the samples if they urinated during the night after going to bed. They were asked to record the times of last urination before going to bed and last urine collection in the morning. The samples were stored in the participants' refrigerators until pick-up in the morning and transported to the laboratory on ice. After mixing and weighing, each urine sample was transferred to three 25-ml disposable plastic tubes and stored at −70°C until analyzed.

Urinary Analysis. Urine samples were analyzed by diode array reverse-phase HPLC for soy isoflavones, their most common mammalian metabolites, and coumestrol (29). In brief, frozen urine samples were thawed, vortex-mixed, and centrifuged at 850 × *g* for 5 min. A 2.0-ml clear supernatant was mixed with 0.4 ml of 0.5 M triethyl-amine acetate buffer (pH 7.0) and 20 μl of flavone (120 ppm in 96% ethanol) as an internal standard. The mixture was incubated with 10 μl of β-glucuronidase (Boehringer # 127680; 200 units/ml, 0.1 μmol/5 μl) and 10 μl of arylsulfatase (Boehringer # 102890; 5 units/ml) for 1 h at 37°C in the dark followed by repeated extraction with diethylether. The combined organic phases were dried under nitrogen and redissolved in 100 μl of methanol by sonication for 10–20 s and in 100 μl of 0.2 M sodium acetate buffer (pH 4.0). After centrifugation, 20 μl of the clear solution were injected into the HPLC system. The internal standard isoflavone was analyzed in the same way in the same batch as urine extracts for internal standard recovery calculation purposes. HPLC analyses were carried out on a NovaPak C18 (150 × 3.9 mm, inside diameter; 4 μm) reverse-phase column (Waters, Milford, MA) coupled to an Adsorbosphere C18 (10 × 4.6 mm, inside diameter; 5 μm) direct connect guard column (Alltech, Deerfield, IL). Elution was performed at a flow rate of 0.8 ml/min with the following step gradient: (*a*) 20% acetonitrile in acetic acid-water (10:90, v/v) for 16 min; (*b*) 70% acetonitrile for 14 min; and (*c*) 20% acetonitrile for 10 min. Analytes were monitored with a dual-channel diode array detector at 260 nm during the entire HPLC run, at 280 nm during equal elution, and at 342 nm during coumestrol elution. Observed peaks were scanned between 190 and 400 nm. These phytoestrogens were identified by comparing retention times and UV absorption patterns with authentic standards analyzed in the same batch and with reported UV data (46). Concentrations of analytes in the urine were calculated with area units obtained from HPLC analyses and the slope of the calibration curve. The excretion rates are expressed in nanomoles/hour after consideration of urine volume and adjustment for the time between last urine collection and previous void and internal standard recovery. Detection limits were 0.2 nmol/h for genistein, 0.4 nmol/h for daidzein and glycitein, 0.9 nmol/h for equol, 1.4 nmol/h for DMA, and 0.5 nmol/h for coumestrol. On 10 randomly selected urine samples, a repeat analysis was performed to test reliability of measurements.

Statistical Analysis. After examining each variable separately for the normality of the distribution, we computed Spearman rank correlation coefficients (47) to evaluate the relationship between urinary isoflavone excretion and dietary intake of isoflavones and soy protein consumption during the previous 24 h and during the previous year. Because of the non-normality of these distributions, we calculated κ-statistics (47) to verify the correlations. A mean coefficient of variation was computed for the 10 urine samples with repeat measurements by dividing the ratio of the SD and the mean of each repeat sample by 10 (47). Nonparametric analyses (47) were performed to evaluate differences among all ethnic groups (Kruskal-Wallis test) and between two ethnic groups (Wilcoxon scores). Statistical analysis was performed using the SAS statistical software package version 6.12 (SAS Institute, Inc., Cary, NC).

Results

The study population included 42 Caucasian women, 25 Japanese women, 13 Chinese women, 11 Native Hawaiian women,

Table 1 Mean soy protein intake and urinary isoflavonoid excretion among 102 women in Hawaii by ethnicity[a]

	Chinese	Filipino	Native Hawaiian	Japanese	Caucasian	Others	Kruskal-Wallis test χ^2 (*P*)
No.	13	7	11	25	42	4	NA[b]
Age (yr)	55 (13)	47 (9)	50 (4)	50 (10)	53 (11)	45 (6)	4.7 (0.45)
Soy protein intake during the previous 24 hours (g/day)	13.3 (18.6)	1.9 (3.4)	8.5 (15.5)	9.1 (11.9)	2.8 (6.6)	7.9 (7.7)	15.1 (0.01)
Soy protein intake during the previous year (g/day)	4.8 (4.4)	1.5 (1.8)	4.6 (3.7)	6.1 (6.8)	1.8 (2.7)	6.6 (4.4)	26.7 (0.0001)
Dietary isoflavone intake during the previous 24 hours (mg/day)	38.2 (56.9)	5.0 (8.8)	22.2 (40.5)	31.3 (43.7)	6.9 (17.6)	17.7 (19.8)	16.0 (0.007)
Dietary isoflavone intake during the previous year (mg/day)	11.9 (11.0)	5.2 (7.5)	12.1 (12.4)	18.9 (27.0)	5.2 (8.6)	16.8 (11.5)	24.3 (0.0002)
Urinary isoflavone excretion (nmol/h)	307.6 (458.7)	77.6 (113.7)	293.7 (689.9)	724.7 (956.7)	138.9 (298.6)	81.1 (134.5)	18.1 (0.003)
Daidzein (nmol/h)	187 (305)	44 (66)	179 (428)	442 (661)	79 (157)	42 (65)	16.2 (0.006)
Genistein (nmol/h)	65 (105)	12 (17)	72 (162)	134 (186)	28 (62)	22 (35)	18.2 (0.003)
Glycitein (nmol/h)	24 (34)	4 (8)	20 (49)	88 (186)	14 (36)	8 (17)	18.7 (0.002)
DMA (nmol/h)	11 (29)	14 (34)	16 (32)	58 (146)	16 (65)	0 (0)	4.0 (0.55)
Equol (nmol/h)	20 (55)	0 (0)	7 (23)	1.8 (9.2)	2 (10)	9 (17)	4.7 (0.45)
Coumestrol (nmol/h)	0 (0)	3 (9)	0 (0)	0.1 (0.5)	13 (70)	0 (0)	3.2 (0.66)

[a] Unless otherwise indicated, mean values are shown, followed by SD in parentheses.
[b] NA, not applicable.

7 Filipino women, and 4 women of other ethnicities (2 African-American women, 1 Indonesian woman, and 1 Vietnamese woman). The age of the study population ranged from 36–80 years, with a mean age of 53 years. The coefficient of variation for the repeat measurements of urinary isoflavone excretion was 9.01%, indicating high reliability of the laboratory procedure.

The mean urinary excretion for isoflavones was more than four times higher in Japanese women than it was in Caucasian women (Table 1; Fig. 1). Urinary isoflavone excretion in Chinese and Native Hawaiian women was more than twice as high as that in Caucasian women. Filipino women and the women of other ethnicities had the lowest urinary isoflavone excretion rate. Whereas only 1 Japanese woman did not excrete any isoflavones, 16 (38%) Caucasian women, 3 (27%) Native Hawaiian women, 1 (25%) woman of other ethnicity, 2 (15%) Chinese women, and 1 (14%) Filipino woman did not excrete any isoflavones. For all ethnic groups, daidzein was excreted at the highest rate, followed by genistein and glycitein (Table 1; Fig. 2). The excretion of urinary isoflavone metabolites and coumestrol showed great variation among ethnic groups (Table 1; Fig. 2). Equol was excreted at the highest rate by Chinese women, whereas all other women excreted equol at a very low rate. Coumestrol was excreted at higher rates by Caucasian women than it was by any other group.

The mean dietary intake of soy protein during the previous year was three times higher for Japanese women than it was for Caucasian women (Table 1). Soy consumption for Chinese and Native Hawaiian women was more than twice as high as that in Caucasian women. Filipino women had the lowest consumption of soy protein during the previous year. A similar pattern of soy protein consumption was obtained for intake during the previous 24 h, except that Chinese women reported the highest soy intake (Table 1).

Assessment by the Kruskal-Wallis test revealed significant differences among ethnic groups for dietary soy and isoflavone intake during the previous year and the previous 24 h (Table 1) as well as significant differences for urinary excretion of daidzein, genistein, and glycitein. The pairwise comparison between various ethnic groups revealed a significantly higher excretion of daidzein by Japanese women than by Caucasian women (χ^2 = 12.6; P = 0.0004) and by Native Hawaiian women (χ^2 = 6.5; P = 0.011). Ethnic differences in the excretion of DMA, equol, and coumestrol were not statistically significant. The ratio of urinary isoflavone excretion to soy intake during the previous 24 h was approximately twice as high for Japanese women as it was for all other women (Fig. 1). Urinary isoflavone excretion showed a strong correlation of 0.61 with dietary soy protein intake during the previous 24 h (Table 2). A weaker but still significant correlation of 0.32 was obtained between urinary isoflavone excretion and self-reported dietary soy protein intake during the previous year. Because dietary soy protein was predominantly obtained from tofu and isoflavone intake was estimated using concentrations in food, the correlation between soy protein and isoflavone intake was greater than 0.9 (Table 2). Excluding observations with nondetectable isoflavone levels did not affect the size of the correlation coefficients. The nonparametric κ-statistic between urinary isoflavone excretion and soy intake during the previous 24 h and during the previous year was 0.46 (95% confidence interval, 0.32–0.59) and 0.23 (95% confidence interval, 0.06–0.42), respectively, confirming the results from the correlation analysis.

Discussion

In this cross-sectional study, we examined urinary isoflavonoid excretion and soy intake in 102 healthy women from different ethnic groups. Japanese, Chinese, and Native Hawaiian women were found to consume higher amounts of soy protein than were Caucasian and Filipino women. Likewise, women with Japanese, Chinese, or Native Hawaiian ancestry excreted more isoflavones in urine than did Caucasian and Filipino women. All of the ethnic groups investigated excreted daidzein at the highest rate, followed by genistein. This is in good agreement with previous reports on urinary isoflavone excretion (39, 40, 48–50). Glycitein was excreted at very low levels, and great variation was observed in the excretion of isoflavone metabolites in all groups. Urinary isoflavone excretion was significantly related to soy protein intake during the previous 24 h and during the previous year as measured by a food frequency questionnaire.

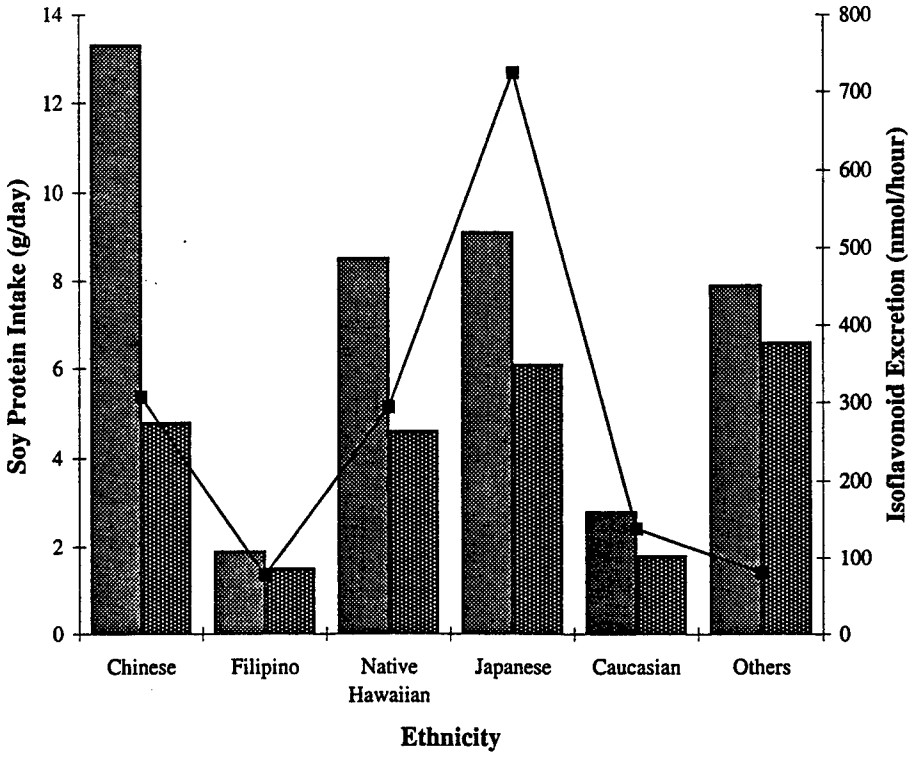

Fig. 1. Mean soy protein intake and urinary isoflavonoid excretion among 102 multiethnic women. □, soy protein intake during the previous 24 h (g/day); ▩, soy protein intake during the previous year (g/day); ■, urinary isoflavones (nanomoles/hour).

Thus far, only a study from California (42) has reported urinary isoflavone excretion by ethnic group. In that study with 50 subjects, no statistically significant difference among various ethnic groups for isoflavone excretion was found, but the small group of Japanese women ($n = 5$) excreted slightly more isoflavones than did other ethnic groups. Similar to our results, Japanese women excreted very low levels of coumestrol compared with Caucasian women. The comparison between the Californian study (42) and our study is limited by the differences in the ethnic groups (Caucasian, Latina, African American, and Japanese *versus* Caucasian, Chinese, Filipino, Japanese, and Native Hawaiian) and the fact that 24-h urine samples were collected in California (42). The advantage of our investigation was a larger sample size (102 women) from a multiethnic population in which women of Asian ancestry have maintained some of their traditional dietary habits.

Other studies with small convenience samples have examined urinary phytoestrogen excretion for various populations, related them to different diets (37, 38), and described a great variation in urinary isoflavones and their metabolites. Urinary isoflavone excretion increased as much as 1000-fold (39) when soy was added to a typical Western diet. Similarly, urinary excretion of isoflavonoids was directly related to dose when 11 men and 9 women consumed soy protein in a controlled experimental diet trial (51) or when randomly added to the usual diet (45). Soy doses (grams of beans) correlated excellently with the observed isoflavonoid amounts excreted in urine during the first 24 h when healthy subjects were challenged with different amounts of roasted soybeans (39).

Inter- and intraindividual differences were reported in a study of six women and five men who consumed 44–96 g of roasted soybeans (39). All individuals excreted genistein and daidzein, but some did not excrete any or excreted only low levels of other isoflavonoid metabolites. A similar variation in the excretion of metabolites and coumestrol among various ethnic groups was obtained in our study (Fig. 1). This variability may be due to the nature of gastrointestinal absorption of soy isoflavones and metabolism by intestinal bacteria (34, 52) such as *Lactobacilli, Bacteroides, Bifidobacterium,* and *Clostridia* (53). Variations in dietary habits can lead to differences in the gut flora (54). The intestinal bacteria vary widely between individuals, and only selected strains are capable of hydrolyzing plant β-glucosides, such as isoflavones occurring in unfermented soy foods, and performing further flavonoid metabolism by ring fission (55, 56). Evidence for that hypothesis was provided in a well-controlled liquid diet study (52) in which seven women consumed three soy milk meals/day. Recovery of daidzein was significantly greater than that of genistein ($P < 0.01$) in women excreting small amounts of fecal isoflavones. Anaerobic incubation of isoflavones with human feces showed that the intestinal half-life of daidzein and genistein is 7.5 and 3.3 h, respectively. Lower absorption levels of isoflavones may explain our finding that Japanese, Chinese, and Native Hawaiian women had relatively high intakes of soy protein, but when compared to Japanese women, excretion of urinary isoflavones was low for both Chinese and Native Hawaiian women (Fig. 1). Variations in the time when soy foods were consumed could also be responsible for this observation.

For this study, only one overnight urine sample was collected for easier implementation and higher compliance, resulting in a participation rate of 78%. Franke and Custer (39) recommended a combination of three urine samples collected every other night for each subject. This would allow integration of an individual's phytoestrogen exposure over approximately 1 week, a period that generally reflects usual dietary habits. Multiple 24-h urine samples would be a more valid measurement of isoflavonoid excreted in urine than one overnight urine sample but would make participation more difficult and costly.

Another limitation of our study was the poor representation of some ethnic groups. The representation of Native Ha-

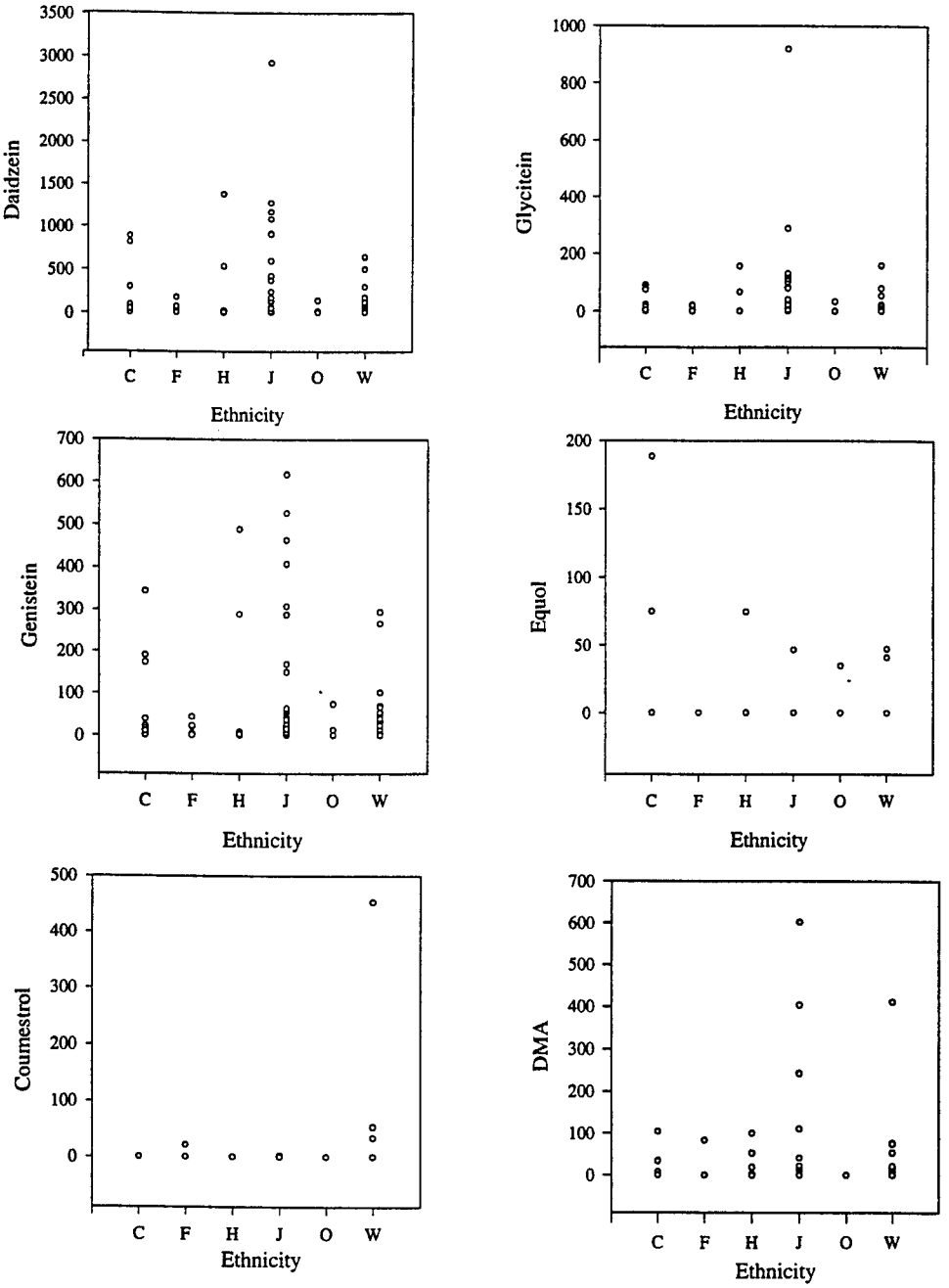

Fig. 2. Urinary excretion of coumestrol, isoflavones, and their metabolites (nanomoles/hour). *C*, Chinese; *F*, Filipino; *H*, Native Hawaiian; *J*, Japanese; *O*, others; *W*, Caucasian.

Table 2 Correlations between urinary isoflavone excretion and dietary intake of soy protein and isoflavones

First variable	Second variable	r_s[a]	*P*
Urinary isoflavone excretion (nmol/h)	Soy protein intake during previous year (g/day)	0.32	0.0012
Urinary isoflavone excretion (nmol/h)	Soy protein intake during previous 24 h (g/day)	0.61	0.0001
Urinary isoflavone excretion (nmol/h)	Isoflavone intake during previous year (mg/day)	0.31	0.0016
Urinary isoflavone excretion (nmol/h)	Isoflavone intake during previous 24 h (mg/day)	0.62	0.0001
Soy protein intake during previous year (g/day)	Soy protein intake during previous 24 h (g/day)	0.53	0.0001
Isoflavone intake during previous year (mg/day)	Isoflavone intake during previous 24 h (mg/day)	0.55	0.0001
Soy protein intake during previous 24 h (g/day)	Isoflavone intake during previous 24 h (mg/day)	0.96	0.0001

[a] Spearman rank correlation coefficient.

waiian women in our study was only 11% as compared with 19% in the population, and the representation of Filipino women in our study was 6% as compared with 11% in the population. Caucasian and Japanese women were overrepresented, probably because of differential insurance coverage and mammography utilization. Language was a barrier for a few women who recently immigrated.

This study is one of the first to investigate soy intake and isoflavone excretion with a larger sample size of healthy women of various ethnic origins. Dietary soy protein and isoflavone intake based on self-reporting for the previous 24 h and for the previous year were positively related to the urinary excretion of isoflavones. The results of this study suggest differences in urinary excretion of isoflavones by ethnic group that are related to self-reported dietary soy intake and to differential intestinal absorption patterns. The strong correlation between urinary isoflavone excretion and self-reported soy intake validates the questionnaire that is now used in a study exploring dietary risk factors for breast cancer.

Acknowledgments

Many thanks go to the staff at the mammography clinics, in particular, to Ginnnie Coggins (Clinical Manager at Kapiolani Women's Center, Honolulu, HI) and Dr. Daniel Henshaw (Kaiser Permanente, Honolulu, HI). We acknowledge Dr. Li-Ching Lyu's expertise in designing the soy food questionnaire and Laurie J. Custer's laboratory skills in performing the isoflavone analysis. Without the time and effort contributed by the participating women, this project would have been impossible. Their participation is greatly appreciated.

References

1. Parkin, D. M., Muir, C. S., Whelan, S. L., Gao, Y. T., Ferlay, J., and Powell, J. Cancer Incidence in Five Continents. IARC Scientific Publ. No. 120. Lyon, France: IARC, 1992.

2. Armstrong, B. K., and Mann, J. I. Diet. *In:* M. P. Vessey and M. Gray (eds.), Cancer Risks and Prevention, pp. 168–198. Oxford, United Kingdom: Oxford University Press, 1985.

3. Hirayama, T. Epidemiology of breast cancer with special reference to the role of diet. Prev. Med., *7:* 173–195, 1978.

4. Nomura, A. M. Y., Lee, J., Kolonel, L. N., and Hirohata, T. Breast cancer in two populations with different levels of risks for the disease. Am. J. Epidemiol., *119:* 496–502, 1984.

5. Buell, P. Changing incidence of breast cancer in Japanese-American women. J. Natl. Cancer Inst., *51:* 1479–1483, 1973.

6. Miller, B. A., Kolonel, L. N., Bernstein, L., Young, J. J. L., Swanson, G. M., West, D., Key, C. R., Liff, J. M., Glover, C. S., and Alexander, G. A. Racial/Ethnic Patterns of Cancer in the United States 1988–1992. NIH Publ. No. 96-4104. Bethesda, MD: National Cancer Institute, 1996.

7. Hankin, J. H. Role of nutrition in women's health: diet and breast cancer. J. Am. Diet. Assoc., *93:* 994–999, 1993.

8. Willet, W. C., Stampfer, M. J., Colditz, G. A., Rosner, B., Hennekens, C. H., and Speizer, F. E. Dietary fat and risk of breast cancer. N. Engl. J. Med., *316:* 22–28, 1986.

9. Zaridze, D., Lifanova, Y., Maximovitch, D., Day, N. E., and Duffy, S. W. Diet, alcohol consumption and reproductive factors in a case-control study of breast cancer in Moscow. Int. J. Cancer, *48:* 493–501, 1991.

10. Adlercreutz, H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand. J. Clin. Lab. Investig., *2014:* 3–23, 1990.

11. Messina, M., and Barnes, S. The role of soy products in reducing risk of cancer. J. Natl. Cancer Inst., *83:* 541–546, 1991.

12. Miksicek, R. J. Estrogenic flavonoids: structural requirements for biological activity. Proc. Soc. Exp. Biol. Med., *208:* 44–50, 1995.

13. Kuiper, G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., and Gustafsson, J. A. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology, *138:* 863–870, 1997.

14. Bennetts, H. W., Underwood, E. J., and Sheir, F. L. A specific breeding problem of sheep on subterranean clover pastures in western Australia. Aust. Vet. J., *22:* 2–12, 1946.

15. Martin, P. M., Horwitz, K. B., Ruyan, D. S., and McGuire, W. L. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology, *103:* 1860–1867, 1978.

16. Shutt, D. A., and Cox, R. I. Steroid and phytoestrogen binding to sheep uterine receptors *in vitro*. J. Endocrinol., *52:* 299–310, 1972.

17. Folman, Y., and Pope, G. S. The interaction in the immature of potent oestrogens with coumestrol, genistein and other utero-vaginotrophic compounds of low potency. J. Endocrinol., *34:* 215–225, 1966.

18. Folman, Y., and Pope, G. S. Effects of norethisterone acetate, dimethylstilboestrol, genistein and coumestrol on uptake of [^{3}H]oestradiol by uterus, vagina and skeletal muscle of immature mice. Endocrinology, *44:* 213–218, 1969.

19. Makela, S., Pylkkanen, L., Santti, R., and Adlercreutz, H. Role of plant estrogens in normal and estrogen-related altered growth of the mouse prostate. *In:* Euro Food Tox III, pp. 135–139. Schwerzenbach, Switzerland: Institute of Toxicology of Swiss Federal Institute of Technology and University of Zanch, 1991.

20. Makela, S., Poutanen, M., Lehtimaki, J., Kostian, M. L., Santti, R., and Vihko, R. Estrogen-specific 17β-hydroxysteroid oxidoreductase type I (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. Proc. Soc. Exp. Biol. Med., *208:* 51–59, 1995.

21. Adlercreutz, H., Bannwart, C., Wahala, K., Makela, T., Brunow, G., Hase, T., Arosemena, P. J., Kellis, J., and Vickery, L. E. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J. Steroid Biochem. Mol. Biol., *44:* 147–153, 1993.

22. Markovits, J., Linassier, C., Fosse, P., Couprie, J., Pierre, J., Saucier, J. M., Le Pecq, J. B., and Larsen, A. K. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res., *49:* 5111–5117, 1989.

23. Ogawara, H., Akiyama, T., Watanabe, S. I., Ito, N., and Kobori, M. Inhibition of tyrosine protein kinase activity by synthetic isoflavones and flavones. J. Antibiot. (Tokyo), *42:* 340–343, 1989.

24. Fotsis, T., Pepper, M., Adlercreutz, H., Hase, T., Montesano, R., and Schweigerer, L. Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and *in vitro* angiogenesis. J. Nutr., *125* (Suppl.)*:* 790S–797S, 1995.

25. Kyle, E., Neckers, L., Takimoto, C., Curt, G., and Bergan, R. Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. Mol. Pharmacol., *51:* 193–200, 1997.

26. Tsukamoto, C., Shimada, S., Igita, K., Kudou, S., Kokubun, M., Okubo, K., and Kitamura, K. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J. Agric. Food Chem., *43:* 1184–1192, 1995.

27. Coward, L., Barnes, N. C., Setchell, K. D. R., and Barnes, S. The antitumor isoflavones, genistein and daidzein, in soybean foods of American and Asian diets. J. Agric. Food Chem., *41:* 1961–1967, 1993.

28. Wang, H., and Murphy, P. A. Isoflavone content in commercial soybean foods. J. Agric. Food Chem., *42:* 1666–1673, 1994.

29. Franke, A. A., Custer, L. J., Wang, W., and Shi, S. J. HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. Proc. Soc. Exp. Biol. Med., *211:* 163–173, 1998.

30. Price, K. R., and Fenwick, G. R. Naturally occurring oestrogens in foods: a review. Food Addit. Contam., *2:* 73–106, 1985.

31. Setchell, K. D. R., and Adlercreutz, H. Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. *In:* I. Rowland (ed.), Role of the Gut Flora in Toxicity and Cancer, pp. 315–345. London: Academic Press, 1988.

32. Joannou, G. E., Kelly, G. E., Reeder, A. Y., Waring, M., and Nelson, C. A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. J. Steroid Biochem. Mol. Biol., *54:* 3–4, 1995.

33. Adlercreutz, H., Fotsis, T., Bannwart, C., Wahala, K., Brunow, G., and Hase, T. Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein. Clin. Chim. Acta, *199:* 263–278, 1991.

34. Setchell, K. D. R., Welsh, M. B., and Lim, C. K. High performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet light, electrochemical and thermospray mass spectrometric detection. J. Chromatogr. B., *386:* 315–323, 1987.

35. Adlercreutz, H., Fotsis, T., Lampe, J., Wahala, T., Makela, T., Brunow, G., and Hase, T. Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography-mass spectrometry. Scand. J. Clin. Lab. Investig., *53:* 5–18, 1993.

36. Franke, A. A., Custer, L. J., Cerna, C. M., and Narala, K. K. Quantitation of phytoestrogens in legumes by HPLC. J. Agric. Food Chem., *42:* 1905–1913, 1994.

37. Adlercreutz, H., Honjo, H., and Higashi, A. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am. J. Clin. Nutr., *54:* 1093–1100, 1991.

38. Goldin, B. R., Adlercreutz, H., Gorbach, S. L., Woods, M. N., Dwyer, J. T., Conlon, T., Boh, E., and Gershoff, S. N. The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women. Am. J. Clin. Nutr., *44:* 945–953, 1986.

39. Franke, A. A., and Custer, L. J. High-performance liquid chromatographic assay of isoflavonoids and coumestrol from human urine. J. Chromatogr. B., *662:* 47–60, 1994.

40. Karr, S. C., Lampe, J. W., Hutchins, A. M., and Slavin, J. L. Urinary isoflavonoid excretion in humans is dose dependent at low to moderate levels of soy-protein consumption. Am. J. Clin. Nutr., *66:* 46–51, 1997.

41. Lu, L. J. W., Lin, S. N., Grady, J. J., Nagamani, M., and Anderson, K. E. Altered kinetics and extent of urinary daidzein and genistein excretion in women during chronic soy exposure. Nutr. Cancer, *26:* 289–302, 1996.

42. Horn-Ross, P. L., Barnes, S., Kirk, M., Coward, L., Parsonnet, J., and Hiatt, R. A. Urinary phytoestrogen levels in young women from a multiethnic population. Cancer Epidemiol. Biomark. Prev., *6:* 339–345, 1997.

43. Hawaii Department of Business, Economic Development and Tourism. State of Hawaii Data Book 1995: A Statistical Abstract. Honolulu, HI: State of Hawaii, 1995.

44. United States Department of Agriculture. United States Department of Agriculture Nutrient Database for Standard Reference. Riverdale, Maryland: United States Department of Agriculture, 1993.

45. Franke, A. A., Custer, L. J., Tanaka, Y., and Maskarinec, G. Isoflavonoids in soy drinks and availability in human body fluids. Proc. Am. Assoc. Cancer Res., *38:* 112, 1997.

46. Franke, A. A., Custer, L. J., Cerna, C. M., and Narala, K. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. Proc. Soc. Exp. Biol. Med., *208:* 18–26, 1995.

47. Rosner, B. Fundamentals of Biostatistics. Boston, MA: PWS-Kent Publishing Co., 1990.

48. Kelly, G. E., Nelson, C., Warin, W. A., Joannou, G. E., and Reeder, A. Y. Metabolites of dietary (soya) isoflavones in human urine. Clin. Chim. Acta, *223:* 9–22, 1993.

49. Ingram, D., Sanders, K., Kolybaba, M., and Lopez, D. Case-control study of phyto-oestrogens and breast cancer. Lancet, *350:* 990–997, 1997.

50. Xu, X., Wang, H-J., Murphy, P. A., Cook, L., and Hendrich, S. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. J. Nutr., *124:* 825–832, 1994.

51. Kirkman, L. M., Lampe, J. W., Campbell, D. R., Martini, M. C., and Slavin, J. L. Urinary lignan and isoflavonoid excretion in men and women consuming vegetable and soy diets. Nutr. Cancer, *24:* 1–12, 1995.

52. Xu, X., Harris, K. S., Wang, H. J., Murphy, P. A., and Hendrich, S. Bioavailability of soybean isoflavones depends upon gut microflora in women. J. Nutr., *125:* 2307–2315, 1995.

53. Gobrach, S. L., Plaut, A. G., Nahas, L., Weinstein, L., Spanknebel, G., and Levitan, R. Studies of intestinal microflora. II. Microorganisms of the small intestine and their relations to oral and faecal flora. Gastroenterology, *53:* 856–867, 1967.

54. Heneghan, J. B. Alimentary tract physiology interaction between the host and its microbial flora. *In:* I. R. Rowland, (ed.), Role of the Gut Flora in Toxicity and Cancer, pp. 39–78. San Diego, CA: Academic Press, 1988.

55. Winter, J., Moore, L. H., Dowell, V. R., and Bokkenheuser, V. D. C-ring cleavage of flavonoids by human intestinal bacteria. Appl. Environ. Microbiol., *55:* 1203–1208, 1989.

56. Miyake, Y., Yamamoto, K., and Osawa, T. Metabolism of antioxidant in lemon fruit (Citrus limon BURM. f.) by human intestinal bacteria. J. Agric. Food Chem., *45:* 3738–3742, 1997.

Abstract Presented at the Department of Defense Breast Cancer Research Program Meeting, Era of Hope, Washington, DC, October 1997 (Poster)

PRELIMINARY RESULTS ON ETHNICITY, SOY, AND MAMMOGRAPHIC DENSITY PATTERNS

Gertraud Maskarinec[1], Li-Ching Lyu[2], Lixin Meng[1], Giske Ursin[3]

[1] Cancer Research Center, University of Hawaii [2]Taipeh Medical College, [3]Norris Comprehensive Cancer Center, University of Southern California

Introduction: Breast cancer risk differs greatly by ethnicity. In 1988-1992, the U.S. breast cancer incidence rates (invasive cases only, age-adjusted to 1970 U.S. population) were $112/10^5$ for Caucasian women, $106/10^5$ for native Hawaiian women, $82/10^5$ for Japanese women, $73/10^5$ for Filipina, and $55/10^5$ for Chinese women. Mammographic density patterns, which refer to the distribution of fat, connective, and epithelial tissue in the healthy female breast, have been shown to be related to breast cancer risk. Therefore, the hypothesis was proposed that women from ethnic groups with high breast cancer risk are more likely to have a dense parenchymal pattern than women from ethnic groups at low risk for breast cancer. Intake of soy products was suggested as one of the dietary factors that may protect Asian women from breast cancer. The purpose of this study was to determine whether mammographic density patterns differ by ethnic background and to explore the possible association of a soy rich diet with mammographic density patterns.

Methods: In this cross-sectional study, healthy postmenopausal women who received a recent screening mammogram were recruited at several clinics in Hawaii. Women with a history of breast cancer or breast surgery were excluded. All study participants completed questions about their medical and reproductive history, a soy food questionnaire, and a validated diet history questionnaire. Body weight was measured on a bathroom scale; body height was self-reported. After scanning the two cranio-caudal mammogram films into a PC, computerized mammographic density assessment was performed. The reader first draws the outline of the breast (using an outlining tool) and then searches for the best threshold gray level value X where all pixels with values above X are considered to

KEYWORDS: Ethnicity, Mammographic Densities, Prevention, Risk Factors, Diet

This work was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-96-1-6284.

represent mammographic densities. The pixel count corresponding to the area colored within the outline of the breast is determined by the computer, as is the total area within the outline of the breast. The proportion of the breast with densities is calculated as the ratio of the colored area to the total area of the breast. The measurements for three readers, whose readings were highly correlated, were averaged. Women with Caucasian (N=16) and native Hawaiian (N=3) ancestry were grouped together. Japanese (N=10), Chinese (N=9), and Korean (N=1) women were included in the Asian group. Student's t-tests and χ^2 tests were applied to assess differences between Caucasian/Hawaiian and Asian women.

Results: So far 39 women have been recruited. Asian women were five years older ($p<0.05$), were more likely to be on estrogen replacement therapy (45% vs. 21%), had a lower body mass index and a three times higher daily consumption of soy protein than Caucasian/Hawaiian women (Table 1). No significant differences in parity, age at first live birth, years of breast feeding, age at menarche and at menopause, educational achievement, daily calories, alcohol intake, and calories from fat were observed between the two groups. The mean area of the breast was nearly twice as large for Caucasian/Hawaiian as for Asian women (Table 1). The mean dense area was also smaller in Asian women, but the difference did not reach statistical significance. In comparison to Caucasian/Hawaiian women, the percentage of densities was slightly higher in Asian women. Excluding women on estrogen replacement therapy did not change the pattern of the results.

Table 1. Results of Mammographic Density Assessment by Ethnicity

Ethnicity	Caucasian/Hawaiian		Asian		p
	Mean	SD	Mean	SD	
Area of Breast (pixels)	201,105	101,798	102,576	61,348	<0.05
Dense Area (pixels)	49,286	27,910	36,519	21,580	0.12
Percent Densities (%)	32%	22%	39%	15%	0.22
Body Mass Index	28.0	6.8	23.4	5.0	0.02
Soy Protein Intake (g/day)	2.3	2.6	7.4	8.6	0.02

Conclusions: These preliminary data suggest that the area of dense tissue in the breast may be smaller in Asian than in Caucasian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Asian women may be equal to or higher than in Caucasian women. We need to perform multivariate analyses in a larger population to investigate the relation between ethnicity, diet, known breast cancer risk factors, and mammographic density patterns.

Abstract Presented at the 2nd Asian-Pacific Congress of Epidemiology, Tokyo, Japan, January 1998

ETHNIC DIFFERENCES IN MAMMOGRAPHIC DENSITY PATTERNS

Gertraud Maskarinec[1], Lixin Meng[1], Giske Ursin[3]
[1] Cancer Research Center, University of Hawaii, U.S.A.
[3]Norris Comprehensive Cancer Center, USC, Los Angeles, U.S.A.

Purpose: Breast cancer risk differs greatly by ethnicity with higher incidence rates among Caucasian and Native Hawaiian women than among Asian women. Mammographic density patterns, which refer to the distribution of fat, connective, and epithelial tissue in the healthy female breast, have been shown to be related to breast cancer risk. Therefore, the hypothesis was proposed that women from ethnic groups with high breast cancer risk are more likely to have a dense parenchymal pattern than women from ethnic groups at low risk for breast cancer.
Methods: In a cross-sectional design, healthy women from different ethnic backgrounds, who were recruited at mammography screening clinics in Hawaii, completed self-administered questions related to medical, reproductive, and diet history. After scanning the cranio-caudal mammogram films into a PC, computerized mammographic density assessment was performed. This method determines the area of the breast with densities and the total area of the breast. The proportion of the breast with densities was calculated as the ratio of the dense area to the total area of the breast. The measurements for three readers were highly correlated. Student's t-tests were applied to assess differences between groups.
Results: The mean area of the breast was nearly twice as large for women with Caucasian and native Hawaiian ancestry as for women with Asian (Chinese, Filipino, and Japanese) ancestry. The mean dense area was considerably smaller in Asian women than in the Caucasian/Hawaiian group. In comparison to Caucasian/Hawaiian women, the percentage of densities was slightly higher in Asian women. Several reproductive and dietary factors as well as hormone replacement therapy were associated with mammographic density patterns.
Conclusion: These preliminary data suggest that the area of dense tissue in the breast may be smaller in Asian than in Caucasian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Asian women may be equal to or higher than in Caucasian women.

Abstract Presented at the 21th Annual Meeting of the American Society of Preventive Oncology. Bethesda, MD, March 1998 (Poster)

A Pilot Study Investigating the Association between High-Density Lipoprotein Cholesterol and Mammographic Densities

G Maskarinec[1], L-C Lyu[2], L Meng L[1], A Theriault[3], G Ursin[4]
[1]Cancer Research Center, University of Hawaii [2]Taipeh Medical College [3]Div. of Medical Technology, University of Hawaii [4]Norris Comprehensive Cancer Center, University of Southern California.

Purpose: To explore the relation between mammographic densities (MD) and high-density lipoprotein cholesterol (HDL-C), which may be a marker of long-term dietary patterns and associated with breast cancer risk, among Asian, Caucasian, and Hawaiian women.
Methods: In this cross-sectional study, postmenopausal women who had received a screening mammogram completed a survey on their medical and reproductive history and usual diet. Using computerized assessment, the total and the dense area of the breast were measured in pixels. The proportion of the breast with densities was calculated as the ratio of the dense area to the total area of the breast. HDL-C measurement was performed on a Kodak Ektachem Analyzer following precipitation of low- and very-low-density lipoproteins.
Results: Mean HDL-C levels were 64 mg/dl in 20 Asian vs. 56 mg/dl in 19 Caucasian/Hawaiian women. Among women who did not take estrogen (N=26), HDL-C was inversely associated with the area of MD (partial R^2=0.15, p=0.03). In the same regression model, daily intake of tofu (partial R^2=0.2, p=0.014), body mass index (partial R^2=0.17, p=0.001), age at menarche (partial R^2=0.06, p=0.06), years of school (partial R^2=0.06, p=0.01), and Asian ethnicity (partial R^2=0.03, p=0.9) were negatively related to densities. Physical activity, dietary fat and alcohol intake were not associated with MD. Substituting the area of MD with the proportion of densities as the dependent variable did not change the variance explained by HDL-C. In women who used estrogen replacement therapy (N=13), HDL-C was not related to MD.
Conclusion: We observed an association between HDL-C and MD in women not taking estrogen replacement, but not in estrogen-users.

Abstract Presented at the 89th Annual Meeting of the American Association for Cancer Research in New Orleans, LA, March 1998 (Poster)

Dietary soy intake and urinary isoflavone excretion among women from a multi-ethnic population. Maskarinec G, Singh S, Meng L, Lyu L-C, Franke A. Cancer Research Center of Hawaii, 1236 Lauhala Str, Honolulu, HI 96813, USA.

Isoflavones are present in soybeans in concentrations up to 300 mg per 100 g, have estrogenic and anti-estrogenic properties, and may be protective against hormone-related cancers. The purpose of this cross-sectional study was to investigate the association between urinary isoflavone excretion and self-reported soy intake. A total of 101 women with Caucasian, Hawaiian, Chinese, Japanese, and Filipino ancestry completed a dietary questionnaire for soy products consumed during the last year and during the 24 hours before urine collection. Overnight urine samples were analyzed for the isoflavones genistein, dadzein, and glycitein and their main metabolites by reversed-phase high-performance liquid chromatography (HPLC). Soy protein and isoflavone intake (predominantly from tofu) were estimated using published nutritional data bases. Student's t tests and Spearman rank correlation coefficients were computed. Asian women consumed 3 times more soy protein than Caucasian/Hawaiian women (8.9 vs. 3.7 g/day, $p=0.03$) and excreted significantly more isoflavones in urine (480 vs. 182 nmoles/hour, $p=0.02$). Dietary soy protein and isoflavone intakes during the previous 24 hours were positively related to urinary isoflavone excretion ($r=0.59$, $p=0.0001$ and $r=0.60$, $p=0.0001$ respectively). Urinary excretion of isoflavones was also related to annual dietary soy protein and isoflavone intake ($r=0.30$, $p=0.002$ and $r=0.29$, $p=0.0024$ respectively). The findings validate measurements of urinary isoflavones as a biomarker for soy consumption, making them a useful tool in future cancer prevention studies. Excretion patterns of specific isoflavones will provide information on bioavailability differences between individuals.

Abstract
Presented at the 1st European Breast Cancer Conference in Firenze, Italy, September 1998

Ethnicity, Diet, and Mammographic Density Patterns

G. Maskarinec[1], L. Meng[1], D. Shumay[1], G. Ursin[2]
[1]*Cancer Research Center of Hawaii;* [2]*University of Southern California*

Purpose: Mammographic density patterns refer to the distribution of fat, connective, and epithelial tissue in the healthy female breast and are strong predictors of breast cancer risk. This project investigated the hypothesis that ethnicity and diet are related to mammographic densities.

Methods: In a cross-sectional design, more than 400 White, Hawaiian, Chinese, and Japanese women with normal mammograms completed a reproductive history and a food frequency questionnaire. After digitizing the cranio-caudal mammographic films, the area with densities and the total area of the breast were measured using a computerized method.

Results: The mean dense area in the mammograms was approximately 15% smaller in Asian than in White and Hawaiian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Asian women was equal to or higher than in White women. In a multiple linear regression model, daily intake of fruits, vegetables, soy products, as well as age, body mass index, age at menarche, and parity were inversely related to mammographic densities, while age at first live birth showed a positive association with densities.

Conclusion: Women from ethnic groups with low breast cancer risk have smaller areas of mammographic densities than women from high risk groups. A diet rich in fruits, vegetables, and soy foods may be related to mammographic density patterns that protect against breast cancer.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

1 JUN 2001

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

DAMD17-94-J-4413	ADB261602
DAMD17-96-1-6112	ADB233138
DAMD17-96-1-6112	ADB241664
DAMD17-96-1-6112	ADB259038
DAMD17-97-1-7084	ADB238008
DAMD17-97-1-7084	ADB251635
DAMD17-97-1-7084	ADB258430
DAMD17-98-1-8069	ADB259879
DAMD17-98-1-8069	ADB259953
DAMD17-97-C-7066	ADB242427
DAMD17-97-C-7066	ADB260252
DAMD17-97-1-7165	ADB249668
DAMD17-97-1-7165	ADB258879
DAMD17-97-1-7153	ADB248345
DAMD17-97-1-7153	ADB258834
DAMD17-96-1-6102	ADB240188
DAMD17-96-1-6102	ADB257406
DAMD17-97-1-7080	ADB240660
DAMD17-97-1-7080	ADB252910
DAMD17-96-1-6295	ADB249407
DAMD17-96-1-6295	ADB259330
DAMD17-96-1-6284	ADB240578
DAMD17-96-1-6284	ADB259036
DAMD17-97-1-7140	ADB251634
DAMD17-97-1-7140	ADB259959
DAMD17-96-1-6066	ADB235510
DAMD17-96-1-6029	ADB259877
DAMD17-96-1-6020	ADB244256
DAMD17-96-1-6023	ADB231769
DAMD17-94-J-4475	ADB258846
DAMD17-99-1-9048	ADB258562
DAMD17-99-1-9035	ADB261532
DAMD17-98-C-8029	ADB261408
DAMD17-97-1-7299	ADB258750
DAMD17-97-1-7060	ADB257715
DAMD17-97-1-7009	ADB252283
DAMD17-96-1-6152	ADB228766
DAMD17-96-1-6146	ADB253635
DAMD17-96-1-6098	ADB239338
DAMD17-94-J-4370	ADB235501
DAMD17-94-J-4360	ADB220023
DAMD17-94-J-4317	ADB222726
DAMD17-94-J-4055	ADB220035
DAMD17-94-J-4112	ADB222127
DAMD17-94-J-4391	ADB219964
DAMD17-94-J-4391	ADB233754