Are World Cancer Research Fund and American Institute for Cancer Research Recommendations for Cancer Prevention Associated with Known Chronic Disease Biomarkers in Healthy Women?

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Abstract

Chronic disease is a leading cause of mortality and morbidity in the United States. Nutritional, lifestyle, hormonal, and other biologic factors are thought to be responsible for the increased chronic disease risk in women. Baseline dietary intake was measured by food frequency questionnaires and repeated 24-hour recalls in 275 premenopausal women. Data were used to evaluate adherence to 10 cancer prevention recommendations as outlined by the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR). The association between adherence and known chronic disease biomarkers was also assessed. WCRF/AICR recommendations were operationalized into eight scores related to body fatness, physical activity, dietary intake, and smoking status. These scores were compared with mammographic density and nipple aspirate fluid as well as biologic markers for chronic diseases found in serum (estrogens, insulin-like growth factor, C-reactive protein and γ-tocopherol) and urine (estrogens and F2-isoprostane).

Results from Analysis of Variance detected weak associations with WCRF/AICR recommendations and the measured biomarkers. Analysis between BMI status and biomarker levels suggested obese women have higher levels of serum C-reactive protein (LS mean of 0.9 mg/L) compared to normal and overweight women (2.3 vs. 4.6 mg/L, respectively; $p<0.0001$). Obese women also had higher γ-tocopherol (LS mean of 1502 ng/mL) compared to normal and overweight women (1777 vs. 1988 ng/mL, respectively; $p=0.02$). Significant positive and inverse associations were also observed between both alcohol and red meat consumption and the majority of the biomarkers measured γ-tocopherol showed the most association of any biomarker with dietary intake. Additional

research is needed in the area of nutrition and biomarkers to understand, which biomarkers may be influenced and how these associations relate to health outcomes. Findings from this study reinforce that biomarkers may be particularly beneficial in understanding the role overweight and obesity plays in the interaction between diet and disease.

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Chapter 1: Introduction

In the past decade the United States has achieved substantial health advances to increase life expectancy for both men and women from 75.2 years in 1990 to 78.2 years in 2010, however many counties across the country saw a disproportionate increase of mortality in women from 1992 to 2006 (1;2). Of the top ten causes of mortality among women, seven were due to chronic diseases such as: heart disease, cancer, stroke, and chronic lower respiratory diseases (3). While there are many risk factors that may explain chronic disease risk, many of these factors are not modifiable. Of those factors that are modifiable numerous recommendations promoting healthier lifestyle and dietary habits have been identified.

One organization, the World Cancer Research Fund & American Institute for Cancer Research (WCRF/AICR), promotes 8 general and 2 specialized recommendations for cancer prevention (4). These recommendations are based on suggestions for chronic disease prevention and a compilation of previous cancer research; including lean body mass, moderate physical activity, minimal consumption of calorie dense foods and drinks, red and processed meats, alcohol and plant based diets. Since a typical diet is not composed of specific nutrients but rather a combination of whole foods, measuring dietary intake using an index, such as the WCRF/AICR, may be beneficial in determining diet-disease relations.

In this analysis, we examined how dietary intake, measured by food frequency questionnaires (FFQs) and repeated 24-hr recalls (24HRs) among 275 healthy premenopausal women related to the WCRF/AICR recommendations for cancer prevention (4). We also analyzed the difference in dietary intake by ethnicity and weight

status and the influence of diet on several biomarkers such as: nipple aspirate fluid (NAF) volume, serum and urinary estrogens, insulin-like growth factor (IGF)-1, and mammographic density for their strong associations with breast cancer risk. Additionally biomarkers, such as F2-isprostane, γ-tocopherol, and C-reactive protein (CRP), may also be associated with increased risk for other chronic diseases (5-8).

Literature review

Dietary recommendations

Numerous health policies and legislative initiatives from various health organizations have been directed at improving health risk factors, e.g. USDA recommendations, for healthful dietary intake. WCRF/AICR, suggested improvements of individual behavior which included: avoidance of tobacco products, maintaining a lean body mass, participating in moderate physical activity, minimal consumption of calorie dense foods and drinks, red and processed meats, and alcohol, with an emphasis on consuming a plant based diet (4). The 8 general and 2 specialized recommendations for cancer prevention, are drawn from the WCRF/AICR second Expert Report, which reviews a comprehensive collection of research and previously published recommendations designed to promote nutritional adequacy to prevent cancer (4).

A recent large European cohort study used 7 of the 10 WCRF/AICR recommendations to create a scoring system. Their results showed that participants who closely adhered to the recommendations had lower mortality than participants who were low adherents (9).

Dietary assessment

Dietary assessments collect self-reported dietary consumption to measure nutrient intake. Two commonly used individual-level food intake assessment methods are FFQs and 24HRs. FFQs are better at capturing long-term usual dietary intake, whereas 24HRs are useful in describing recent dietary intake (10).

Although, FFQs are more commonly used for large cohort studies, individual dietary data obtained from a structured questionnaire tend to have large measurement errors and higher reporting of intakes compared to 24HRs (11). A previous study that used doubly labeled water and urinary nitrogen as biomarkers for total energy expenditure and protein intake showed that women underreported FFQ and 24HRs energy compared with total energy expenditure (12). They also underreported protein (12). A review of several studies indicated that under-reporting is common in overweight and obese individuals and suggests that people will report intakes close to their cultural or population norms (13).

Even with FFQ's vulnerability to systematic and random error, e.g. underreporting, they are inexpensive and constitute a practical dietary assessment tool for nutritional epidemiologic studies. Therefore, validation and calibration of the FFQ is important to optimize statistical tests for diet-disease associations by obtaining accurate intake (14). One way to validate dietary assessment is through use of reference instruments, such as 24HRs and biomarkers. The European Prospective Investigation into Cancer and Nutrition Cohort (EPIC) in ten European countries used questionnaire measurements, records of daily intake, and biochemical markers as references for

validation (14), whereas, the Multi-Ethnic Cohort (MEC) used repeated 24-hr recalls (15).

Ethnic difference in dietary intake

Hawaii's population is unique because of its diverse ethnic groups, mainly comprised of: Asians, Native Hawaiians, and Whites. From the population composite, a distinct food culture developed in Hawaii incorporating traditional Asian and Native Hawaiian dishes with Western style cooking. Current generations have a unique consumption of food groups and nutrients based on local food culture and ethnic specific dietary preferences (16).

A previous study based on the MEC measured the ethnic differences of total energy, fiber and percent fat intake between 5 ethnic groups; African American, Caucasian, Latino, Native Hawaiian, and Japanese American (17). The study observed that Native Hawaiian women had the highest mean total energy intake and the lowest fiber consumption. Caucasian and Japanese American women had similar means for total energy, which were also the lowest. Japanese American women also consumed the least fat by percent of energy.

Another study using factor analysis to identify distinct dietary patterns in multiethnic women also saw a low energy and fat intake among Chinese and Japanese participants. Of the dietary patterns observed, the "meat" pattern, characterized by high amounts of red meats, fish, poultry, eggs, fats, and condiments, was more frequently observed for Native Hawaiians compared to women of other ethnicities.

Dietary intake and weight status

A typical diet is not composed of specific nutrients but rather a combination of whole foods. For many dietary intakes are habitual and patterned. Measuring dietary intakes and patterns are useful for exploring relationships with BMI. In turn, obese and overweight US adults are at increased risk for major diseases, such as breast cancer and other chronic diseases (18;19).

Although dietary determinants of weight gain are not fully understood, several studies have observed a lower BMI associated with dietary patterns that are high in fiber-rich foods and low in fat (20;21). A study of 5292 participants living in the UK observed that a higher proportion of meat eaters than non-meat eaters were overweight or obese. They also observed a significant inverse association between fiber intake and BMI and a positive association with animal fat intake and BMI status (22). Other studies did not find significant differences between fiber, fat and BMI status with dietary pattern, but did observe that energy density of foods consumed was positively associated with body weight (17).

Biomarkers associated with increased breast cancer risk

Breast cancer is the second most common cause of cancer death among women. Nutritional and lifestyle factors, hormonal and other biologic factors are associated with increased breast cancer risk. Biomarkers are biologic measures that predict risk of cancer and other chronic diseases. In addition, they can help in early detection or to evaluate individual risk for disease development. Serum and urinary estrogens, nipple aspirate

fluid (NAF), mammographic density, and serum insulin-like growth factors (IGF)-1 are common biomarkers that are associated with breast cancer.

Female sex hormones, estrone (E_1) and estradiol (E_2), have long been associated with development of breast cancer. They affect the growth of estrogen sensitive cells like the mammary gland and women who are exposed to elevated levels for long periods are found to be at increased risk for cellular mutations and uncontrolled division (23). Age is an important factor in breast cancer development, but dietary intake may also modify sex hormone concentrations.

One meta-analysis, based on the review of 13 studies, examined the effects of dietary fat interventions on serum estrogen levels (24). Fat intake in the range of 10-25% of total energy consumed, was associated with a statistically significant decrease of serum E_2 in both premenopausal and postmenopausal women (24). One sub-analysis of the meta-analyses observed a decrease of about 20% in serum E_2 after 2 years of a low fat high-carbohydrate diet in premenopausal women. Effects of the intervention were seen before and after controlling for the day of menstrual cycle on which blood was collected (25). Another study observed drinking more than one serving of alcohol per day was associated with higher E_2 levels (26).

Although not significant, trending results from one study suggest that women who consumed a vegetarian/pescatarian based diet rather than an omnivorous one had lower serum E_1 and E_2 levels (27). Another study suggests a Western dietary pattern, characterized by higher intakes of red and processed meats, refined grains, sweets and desserts, was associated with higher serum E_2 (28). However, after controlling for BMI,

only an inverse association between E_2 and the Alternative Healthy Eating Index was significant. (28).

NAF is another minimally invasive biomarker of increasing interest in breast cancer research. The volume of NAF production varies by individuals but four factors are associated with NAF production: age of 35-50 years, early menarche, history of parity and lactation, and non-Asian ethnicity (29). NAF volume and especially abnormal epithelial cytology is associated with women at high risk for breast cancer, i.e., family history of breast cancer, compared to women without those risk factors (30).

A modifiable risk factor associated with NAF volume is dietary fat and saturated fat intake. In one study, women capable of producing NAF had a greater mean intake of both fat and saturated fat compared to non-producers (29). Specifically, white women who had ever smoked were also more likely to yield NAF (29). Although, epithelial cells are not always detected in NAF, another study observed an increase of epithelial cells in NAF with increasing total fat intake (31). The same study observed weak inverse associations with fiber, fruit, and vegetables (31).

Similar to estrogens and NAF-volume, recent studies also observed inconsistent associations between dietary intake and mammographic density. Mammographic density represents the amount of stromal and glandular tissue in the female breast (32). Women with dense breast tissue are at a greater risk of developing breast cancer compared to women with little or no density (32). Breast density decreases with age but some studies associate a decrease in density from diet intake as well (32).

One study recruited premenopausal women for a two-year low-fat, high-carbohydrate diet (33;34). In the initial intervention, a significant decrease in

mammographic density was observed for women consuming a low-fat diet and undergoing natural menopause (33). The follow up study with a larger cohort, observed only menopause as predictor with a significant inverse association (34). However, another study suggests that breast density is higher with lower intake of saturated fat in premenopausal women (5). In the same study, alcohol was linearly associated with mammographic density in both premenopausal and postmenopausal women (5).

The peptide hormone IGF and IGF binding proteins (IGFBPs), which can enhance or inhibit IGF effects, are responsible for maintaining lean mass, glucose regulation, growth, organ development and protein synthesis (35;36). Higher plasma levels of IGF and IGFBPs are associated with both positive (bone density, muscle growth) and negative (increased cancer, diabetes) health outcomes (35). Several studies have suggested a link between IGF-1 and its binding proteins (IGFBP-3) with nutritional factors and mammographic density (37;38). Of special interest of the IGF signaling pathway is the IGF-1 receptor (IGF-1R), where over-expression and activation by phosphorylation is associated with the transformation of cells, cancer cell proliferation, and metastases (39).

Recent findings suggest habitual moderate alcohol consumption decreases IGF-1R phosphorylation and IGF-1 concentrations (35). Cruciferous vegetables, like broccoli, are also associated with lower IGF-1 concentrations and IGF-1R activation (40). Other studies found that total energy and protein intake were positively associated with plasma IGF-1 levels; IGF-1 levels decreased during caloric restriction (41).

Oxidative and inflammatory markers

An imbalance between anti-oxidants and free radicals may cause oxidative stress, and interfere with normal biochemical functions. Continued oxidative stress is associated with chronic inflammation and can lead to DNA damage. Oxidation products, such as F2-isoprostanes in blood and urine, are reliable and sensitive markers of oxidative stress (42). F2-isoprostanes are a measure of lipid oxidation that appear to be specifically associated with cellular nitric oxide production, and elevated levels are associated with heart disease, inflammation, and cancer risk (42).

Cigarette smoking and dose-dependent alcohol consumption are associated with elevated F2-isoprostane levels in both urine and plasma (43). High dietary intake of fruits, and vegetables, with high levels of antioxidants, are associated with lower levels of F2-isoprostanes in adolescents (44). One study observed lower F2-isoprostane concentrations for a fruit and vegetable based diet, and a diet, characterized as adhering closely to USDA dietary guidelines (45). Of the two inverse associations, the plant-based diet had the lower mean concentration levels of F2-isoprostanes (45).

As observed for F2-isoprostane, the antioxidant-rich nature of a plant-based diet is inversely associated with CRP levels (46). CRP is an acute-phase protein that is released by the liver and stimulated by inflammation, infection, and tissue damage (46). CRP serum levels are moderately elevated during chronic inflammatory diseases, such as cardiovascular disease and cancer (47). Many associations found between dietary and lifestyle factors for F2-isoprostane were also detected for CRP. Moderate habitual alcohol consumption has an inverse association with CRP levels (48). Lower concentrations of

CRP were reported in individuals involved in high physical activity (49). The opposite is observed for obesity, CRP levels increase with higher BMI (49;50).

Other potential biomarkers that are affected by chronic inflammation and oxidative stress are tocopherols, comprised of α-tocopherol (Vitamin E) and γ-tocopherol (51). Tocopherols are known for their antioxidant function, and may reduce damage caused by chronic inflammation and oxidative stress through scavenging of free radicals (51). One study suggests that nut consumption has a protective effect against disease while vitamin E supplementation showed no association (7). Vitamin E supplements are mostly comprised of α-tocopherol which at high doses can lower γ-tocopherol levels, where as consumption of Vitamin E from a balanced diet helps maintain optimal levels of both (7). Serum levels of α-tocopherol are associated with dietary intake of vitamin E rich foods and inversely with BMI. In contrast γ-tocopherol levels are not associated with dietary intake and higher BMI in women was positively associated with higher serum levels of γ-tocopherol (50). γ-tocopherol in blood is also positively associated with many other inflammatory conditions and markers, such as smoking, CRP, vitamin D deficiency and colorectal cancer (7). Apparently, γ-tocopherol, unlike α-tocopherol, possesses anti-inflammatory properties and its rise in plasma in response to inflammatory stimuli may be a response by the body to limit damage associated with inflammation, making it a unique antioxidant biomarker (7;52).

Research objectives and hypothesis

The objectives of this project were 1) to compare the nutrient intakes of baseline data as assessed by FFQs and 24-hr recalls of healthy premenopausal women; 2) to create

a scoring system to assess adherence to a healthy lifestyle index modeled after the American Institute of Cancer Research dietary recommendations; 3) to explore the differences in dietary intake and adherence to the lifestyle index across ethnic groups and BMI status; 4) and to examine the association of dietary intake and adherence to the lifestyle index with several biomarkers of chronic disease. We hypothesize that women who closely adhere to the healthy lifestyle index and/or consume a diet rich in plant foods and low in alcohol, added sugar, and meat have more favorable levels of chronic disease biomarkers.

Chapter 2: Methods

Study information

Study design and population

Our analyses used baseline data from two previous intervention studies: the original Breast, Estrogen, and Nutrition (BEAN1), which randomized 220 women to a 2-year clinical trial to examine the effects of 2 daily soy servings on sex steroids and mammographic densities and BEAN2, which was a cross-over design, where 82 women completed a 6-month high-soy and a 6-month low-soy diet with a 1-month washout between diets (53;54). The protocols for both studies were approved by the University of Hawaii Committee on Human Studies and by the institutional review boards of the participating hospitals. All participants signed an informed consent form before entry into the trials. A Data Safety Monitoring Committee reviewed the progress of the study, reasons for dropouts, and any reported symptoms.

For BEAN1, 10,022 women were invited to participate in the trial, 975 replied, 352 were eligible and 245 participated in a run-in period of 1 week (53). The BEAN2

trial invited 16,306 women to participate, 825 replied, 310 were screened, 96 were randomized, and 14 dropped out of the study (55). Eligibility criteria for both studies included a normal mammogram, no breast implants, no oral contraceptives, not pregnant, no previous cancer diagnosis, intact uterus and ovaries, regular menstrual cycles, and low soy intake (55). Additional criteria for BEAN2 included the ability to produce at least 10 μL NAF, one of the study outcomes (54).

After exclusion of dropouts and women with incomplete data, the current analysis included 275 women who provided biological samples and complete nutritional information from baseline FFQs and 24HRs.

Study procedures

The same dietary intervention protocol was used in both studies; the high soy diet consisted of 2 servings of soy foods providing approximately 50 mg of isoflavones per day. During the low soy diet of the two BEAN studies, participants were encouraged to continue their regular diet and were counseled how to minimize soy intake (55).

Data collection

Demographic and dietary information

All participants completed a baseline FFQ that included information on habitual dietary intake during the last year. There was a single questionnaire for all ethnic groups that used eight frequency categories for foods and nine for beverages. The highest frequency category for food items was ≥2 times/day and ≥4 times/day for beverages. Respondents could choose from three typical serving sizes and photographs were used to

help visualize proportions for selected foods. Information was also collected for condiments and additions to breads, coffee, and tea. Types of fats and oils used for cooking and usual practice of eating fat on meat or skin on chicken were also noted (15).

The FFQ used in our study was borrowed from the MEC, which was calibrated with three 24HRs and two FFQs (15). The correlations for regression of mean 24HR intakes on the FFQ intakes by ethnic-sex group for 10 selected dietary components: total calories, protein, fat, saturated fat, carbohydrate, calcium, vitamin A, vitamin C, β-carotene, and dietary fiber, observed strong correlations with nutrient densities than absolute values of nutrients (15). The highest average correlations for absolute intake were white males and females (R=0.57 and 0.48) and the lowest were African-American males and females (R=0.30 and 0.26) (15). All groups had relatively high average correlations for nutrient densities. The lowest was 0.57 in Latino females and the highest of 0.74 in White females (15).

Other demographic data was collected with the same questionnaire packet as the FFQ. Self reported data included questions about physical activity, smoking status, medical history that included use of oral contraceptives and also questions on reproductive characteristics such as first and last pregnancy and number of children. BMI was calculated from weight and height measured at baseline. The participants self-reported their ethnic background and were instructed to include all ethnic backgrounds that applied.

24-hr dietary recalls

To assess adherence to the study protocol, all participants completed 7 unannounced 24HRs. In BEAN1, all recalls during the 2-year period were conducted by telephone, whereas in BEAN2, trained staff collected the first recall during the screening visit and 3 recalls during each diet period by telephone (53;54). The 24HRs used standardized protocols, standard probes, and a 3-pass method to obtain a detailed account of all foods and beverages consumed during the previous day. The dietitian inquired about preparation methods and additions and probed about easily forgotten foods. Weekdays and weekend days were included. Given the long duration of the studies, seasonality of foods was not a problem; all women had a chance to report their diet during different parts of the year.

The FFQs and the 24HRs were analyzed utilizing the Food Composition Table maintained by the Nutrition Support Shared Resource at the Cancer Center (56); the databases represent an extensive list of local foods consumed by the various ethnic populations of Hawaii and the Pacific.

Estrogens and metabolites in urine and serum

Serum and urine samples were collected during the midluteal phase using ovulation kits in BEAN1 and confirmation by serum progesterone levels (53) and self-reported menstruation information in BEAN2 (53;54). All specimens were stored at -80ºC after aliquoting. Using validated radioimmunoassay (RIA), 5 repeated serum samples for BEAN1 and 3 samples for BEAN2 were analyzed for E_1 and E_2 in 0.5 mL serum (57). Based on blinded samples, the interassay coefficient of variation (CV) were 17.7% for E_1 and 11.2% for E_2 in BEAN1 and 15.0% for both in BEAN2 (53;54).

In both studies, repeated overnight urine samples were collected in containers with added ascorbic and boric acid to control bacterial growth (55). For BEAN1, the baseline samples were analyzed for 173 women after 10-13 years of storage and baseline samples for BEAN2 were analyzed after 1-4 years of storage. The predominant steroidal estrogens and metabolites in premenopausal women, namely E_1, E_2, 2-OHE_1, 2-OHE_2, 2-$MeOE_1$, 4-OHE_1, E_3, 16keto-E_2, 16α-OHE_1, were measured by liquid chromatography mass spectrometry (LCMS) (model Exactive, Thermo Fisher Scientific, Waltham, MA) using 5 labeled internal standards as described previously (58;59). Ascorbic acid was added during hydrolysis and during derivatization to prevent artificial oxidation of sensitive analytes. Analysis of an external urine pool from premenopausal women repeated on 9 different days revealed CVs of 4-21% depending on the analyte concentrations. Urinary creatinine concentrations were measured using a Roche-Cobas MiraPlus clinical chemistry autoanalyzer (Roche Diagnostics, Switzerland). Urinary values were expressed relative to Mg creatinine to adjust for urinary volume.

Mammographic density

The participant's physicians ordered mammograms and none were performed specifically for the BEAN1 study. Personal information was removed and baseline images of both breasts were scanned by Kodak LS85 Film Digitizer (Kodak, Rochester, NY). A researcher performed computer–assisted density assessments.

The mammographic measures included the total breast area, the dense area of the breast, and the percentage density, calculated as the ratio of dense area to the total area. The values were averaged for the left and right breast except for two women for whom

only one side was available at baseline. The two readings were strongly related; the Pearson correlation coefficients varied between 0.92 and 0.97 for the mammographic measures of interest. A sample of 219 mammograms was read twice to determine reproducibility. The intraclass correlation coefficient (ICC) was ICC = 0.95 (95% CI: 0.93-0.96) for the percentage density. Mammograms were collected from the 98 intervention and 103 control women. Only 6 women from each group had final mammograms obtained at 12 months because they left before the study concluded. An additional 7 control and 10 intervention women only had baseline mammograms (60).

Serum insulin-like growth factor

IGF-1 was measured by double-antibody enzyme-linked immunosorbent assay (ELISA) assays (Diagnostic Systems Laboratories, Webster, TX, USA) (61). Each BEAN1 subject's samples were analyzed in the same batch. Batches were assembled with an equal number of intervention and control samples and when possible balanced according to ethnicity and age. The assay quality was assessed with 60-blinded quality controls from a pooled serum sample. The laboratory standard range for inter-batch CV was 12.5-16.1% and the IGF-1 mean inter-batch CV was 15.4%,. The Pearson correlation coefficients for IGF-1 samples from a previous study using the same methodology as the one used in BEAN1 was 0.82; this demonstrates its high reproducibility (37;62).

Serum C-reactive protein

The BEAN1 serum level of CRP was assessed by a latex particle enhanced immunoturbidemetric method using a Cobas MiraPlus Clinical autoanalyser and a kit

from Pointe Scientific, Inc., Lincoln Park, MI. Batches composed of 30 or 40 samples contained all samples of each woman and an even number of intervention and control group. The detection limit was 0.1 mg/L (63). The measurement quality was assessed by 49 blinded controls from a pooled sample donated by 10 premenopausal center employees. The mean intra-batch CV was 6.1% and the inter-batch CV was 18.2% (63).

Serum γ-tocopherol

Serum samples were analyzed for γ-tocopherol by reverse phase high pressure liquid chromatography (HPLC) with photodiode array detection between 220 – 600 nm. A mixture of 0.30 ml of serum and 0.30 ml of ethanol containing butylate hydroxytoluene as antioxidant and tocol internal standards, was followed by dividing into 0.8 ml hexane. The hexane layer was evaporated in amber vials at room temperature under a stream of nitrogen. The dry extracts were re-dissolved in 0.15 ml HPLC mobile phase. The separation for tocopherols was performed on a Spherex ODS analytical and guard column with a mobile phase consisting of MeOH, CHcl2, MeCN, bis-tris propane, and butylated hydroxytoluene. The assay was regularly validated during the analysis of external standards within each sample batch and through participation in the quality assurance program organized by U.S. National Institute of Standards and Technology (Gaithersburg, MD) (50;64).

Nipple aspirate fluid

Trained staff demonstrated the collection technique using a FirstCyte Aspirator, for NAF collection, in BEAN2. The samples were carefully collected in microcapillary

tubes and the total amount was recorded to the nearest μL from each woman at baseline. The collections were attempted during the mid-luteal phase (3-11 days before the next menstruation) based on self-reported information on previous menstruation dates, cycle length and actual date of next menstruation. Due to time constraints, only 53% of the baseline NAF samples were collected during the mid-luteal phase. The intra-class correlation coefficient of NAF volume of 0.58 over 7 NAF collections throughout the study indicated stability of NAF volume within individuals across time (54;65).

Urinary F2-isoprostane

Urinary F2-isoprostane were measured using an ELISA kit (Oxford Biomedical Research, Rochester Hills, MI). Over a course of 6 days, 6 batches were run according to manufacturer's instructions, 38 samples for 13 women on Plate 1, 39 samples for 13 women, on Plate 2, and 42 samples for 14 women each on Plates 4-6. To increase accuracy of the test, thawed urine samples were treated with β-glucoronidase from a glucoronidase sample prep kit (Oxford Biomedical Research, Rochester Hills, MI) before undergoing ELISA, since isoprostanes excreted in human urine are often conjugated to glucoronic acid. Batches 1-2 used 8 μl of glucoronidase for 200 μl of urine while batches 3-6 used 8 μl for 100 μl of urine.

Adjustments were made for the variation in method during the statistical analysis. A generated standard curve using a 4-parameter fit from the plot of concentration and absorbance was used for quantification of isoprostane concentrations. The minimum limit of detection was 0.1 ng/ml. With the exception of batch 3, each plate contained at least one quality control sample from a pool of urine. The intra-assay and interassay

coefficient of varations were 7.9% and 22.2%, respectively. Creatinine levels were analyzed with a Roche-Cobas Mira Plus (Crumlin, UK) that is based on a kinetic modification of the Jaffe reaction. F2-isoprostane excretion was expressed as nmol/mg creatinine to adjust for urine volume (66).

Statistical analysis

Baseline statistical analyses were performed using SAS, release 9.4 (SAS Institute, Inc., Cary, NC). All analyses and p-values were considered statistically significant at α=0.05. The correlation analysis between the FFQ and 24-hr recall dietary assessments were carried out using Spearman rank correlation coefficients. Food servings were defined according to the Food Guide Pyramid (67).

A scoring system was created modeled after 8 of the 10 recommendations by the WCRF/AICR for cancer prevention. Participants were given a score of 1 or 0 depending on if they met or did not meet a recommendation (Table 3). The new score variables were created using if, else if, and then statements. WCRF/AICR categories of adherence were determined as low (<5), moderate (=5 to 6), and high ($\geq$7) based on the number of recommendations met. The three categories were created to help observe if statistically significant differences existed between extremes, low and high adherence, with moderate containing the majority of women.

Analyses for BMI status were based on pre-determined BMI categories, normal (<24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (>30 kg/m^2). We did not separate underweight (<18.5) because only three women had such a low weight and removal did not affect statistical significance.

Dietary and lifestyle habits were stratified by BMI, ethnic group, and adherence groups, whereas adherence to WCRF/AICR recommendations was stratified by BMI and ethnic groups, and biomarkers were stratified by BMI and adherence group. The analysis was conducted by Analysis of Variance with the GLM procedure. Non-normally distributed variables were log transformed to obtain p-values for trend tests. The association between the categorical components within the WCRF/AICR score and categorical BMI and ethnicity were analyzed using Chi-square test and likelihood ratios. To determine the significance of ethnic differences was accomplished by Tukey's range test.

We did not make adjustments when comparing dietary intake between low, moderate, and high adherence groups because there was not significant change after including total energy intake as a covariate. However, we adjusted for age and BMI when comparing dietary intake by ethnicity. Adjustments for total energy intake and age were made when we compared dietary intake and lifestyle patterns by BMI status. Adjusted differences were expressed as least square means with 95% confidence intervals, whereas unadjusted results were expressed as means and standard deviation. Analyses with biomarkers were adjusted for age, parity, luteal phase, ethnicity, and total energy intake.

Chapter 3: Results

Description of study population

The multi-ethnic population of our study (Table 1) was primarily composed of Whites (41%), followed by Asians (36%), Native Hawaiians (14%), and all other ethnic groups were classified as Others (9%). The mean age of women was 41.9 years with a

minimum of 24 and maximum of 50 years. The mean height of participants was at the national average of at 1.6 (68) meters with a mean weight of 68.2 kg resulting in a mean BMI status of 26.1 kg/m^2. A majority of the participants never smoked (65.1%), with 28.7% still currently smoking and 6.2% as past smokers. Most women had given birth with a mean of 2.2 children and only 71 (25.8%) were nulliparous.

Dietary and lifestyle habits

Dietary assessments

We computed correlations between FFQs and 24-hr recalls for commonly consumed foods and specific intakes used as part of WCRF/AICR dietary recommendations (Table 2). Sodium had the highest correlation between FFQ and 24-hr recall (mean intake 2781±1469 vs. 2541±764 mg/day respectively; r=0.74). Interestingly, red meat was also highly correlated (mean intake 1.3±1.1 vs. 1.2±1.0 servings/day respectively; r=0.51). Other significant correlations ≥ 0.4 were observed for alcohol (r=0.55) and percent fat (r=0.46). The lowest correlations were found for vegetables intake (r= 0.05) and fruits with vegetables intake (r=0.10), which were also the only two non-significant correlations observed. We typically saw higher means and standard deviations for FFQs compared to 24HRs, such as total energy intake (1911±893 vs. 1683±379 kcal/day), fiber (20.1±11.6 vs. 16.2±5.4 g/day), and sodium (2781±1469 vs. 2541±764 mg/day).

Adherence to WCRF/AICR recommendations

The total score achievable for our healthy lifestyle scoring system modeled after WCRF/AICR recommendations was 8 (Table 3). For individual constituents, most participants followed the recommendation for drinking 1 or less alcoholic beverages per day (90.2%) followed closely by low red meat consumption (88.4%). The least followed recommendation was to eat vegetables, fruits, and whole grains; 188 (68.4%) participants did not comply. Although most women met the recommendations for physical activity (64.7%) and low consumption of energy dense foods (71.3%), only a little more than half met the body fatness recommendation (50.2%). Only 132 (48%) adhered to the recommendation of limiting sodium intake to less than 2400 mg/day.

Group comparisons

Characteristics by low, moderate, and high WCRF/AICR adherers

The 72 women considered low adherers, women who met less than 5 of the healthy dietary and lifestyle index recommendations, had a mean BMI of 30.3±6.0 kg/m^2 (Table 4), while, the mean BMI of the 53 high adhering women was 22.2±2.0 kg/m^2.

Comparison of food intake between adherence groups indicated that those in the high adherence group consumed less meat (0.9±0.7 vs. 1.2±1.1 vs. 1.7±1.0 servings/day; $p<0.0001$), percent fat (30.0±5.6 vs. 33.0±5.8 vs. 33.6±6.3 %; $p=0.002$), and sodium (2163±834 vs. 2811±1622 vs. 3171±1364 mg/day; $p<0.0001$), and had more fiber (22.9±10.0 vs. 20.1±13.1 vs. 18.1±8.8 g/day; $p=0.02$), fruits (1.9±1.3 vs. 1.5±1.8 vs. 1.2±1.1 servings/day; $p=0.005$), and fruits with vegetables (5.5±2.5 vs. 4.9±4.0 vs. 4.1±2.5 servings/day; $p=0.01$) in their diet. High adherers were also more physically

active (12.1±9.9 vs. 7.4±6.1 vs. 5.1±8.4 hrs/week; p<0.0001). As expected, we noticed a significant difference between adherence groups for intakes and some life style factors that were part of the scoring system, with the exception of alcohol and whole grains. Significant trends were also observed for vegetables and regular soda intake.

Characteristics by ethnic group

Table 5a shows several differences between ethnic groups. Asians had the lowest BMI of 24.5 kg/m^2 (95% CIs [23.4, 25.6]), which was significantly different from both Whites (26.6 kg/m^2, 95% CIs [25.6, 27.6]; p=0.03) and Native Hawaiians (28.8 kg/m^2, 95% CIs [27.0, 30.5]; p=0.0005). Significant ethnic differences in dietary intake were observed for meat (p=0.0008), fiber (p=0.03), fruit (p=0.006), fruits with vegetables (p=0.02), and alcohol (p=0.006). Whites consumed the least amount of red meat (1.1 servings/day, 95% CIs [0.9, 1.3]), the most dietary fiber (21.9 g/day, 95% CIs [19.7, 24.0]), fruits (1.7 servings/day, 95% CIs [1.5, 2.0]), and fruits with vegetables (5.3 servings/day, 95% CIs [4.7, 5.9]).

The ethnic groups defined as Other, all other ethnic groups not captured by White, Asian, or Native Hawaiian, were on the opposite end of the spectrum and consumed the least amount of fiber (18.4 g/day, 95% CIs [14.0, 22.8]), fruits (1.1 servings/day, 95% CIs [0.5,1.7]), and fruits with vegetables (4.0 servings/day, 95% CIs [2.7, 5.3]), but Native Hawaiians reported about half a serving more red meat than Whites (1.6 servings/day, 95% CIs [1.3, 2.0]). As expected, each ethnic group consumed less than 1 drink of alcohol per day, but there was still a significant difference between ethnic groups. Asians consumed the least amount of alcohol, 0.2 drinks per day (95% CI [0.1,

0.3]), followed by Native Hawaiians and Others who both consumed 0.3 (95% CIs [0.1,0.5]) drinks per day, while Whites consumed the most with 0.5 drinks per day (95% CIs [0.4,0.6]; p=0.006).

Self-recorded physical activity also differed significantly between ethnic groups, but Others (9.5 hours/week, 95% CIs [6.5, 12.5]) had more active hours per week than Whites (8.9 hours/week, 95% CIs [7.4, 10.3]). The lowest hours of exercise a week were observed for Native Hawaiians (6.5 hours/week, 95% CIs [4.0, 9.1]) and Asians (6.4 hours/week, [4.9, 8.0]; p=0.0008).

Of the 8 individual healthy lifestyle index recommendations, only two were significantly different by ethnicity (Table 5b). As expected from data provided in Table 5a that show significant differences in mean BMI (p=0.002) and hours of physical activity (p=0.0008), we observed corresponding differences for those two parts of the healthy lifestyle score. Asians had the highest percentage (61.6%) that met the recommendation and the lowest mean BMI, whereas Native Hawaiians had the lowest adherence (26.3%) and the highest mean BMI. Physical activity had a similar pattern with Whites and Others having the highest adherence and the most physical activity hours per week.

While there were significant differences in red meat, fruit with vegetable, and alcohol consumption in Table 5a, we did not see the same pattern in Table 5b for the comparable lifestyle scores. Even with only two individual scores being significantly different, the mean for the total score still differed significantly between ethnic groups (p=0.007). Whites had the highest mean WCRF/AICR adherence score and Native

Hawaiians the lowest (5.6±1.3 vs. 4.8±1.2; p=0.003) and Native Hawaiians were also significantly different from Asians (4.8±1.2 vs. 5.4±1.3; p=0.05).

Characteristics by BMI status

When women were grouped by BMI status, mean values differed between normal, overweight, and obese BMI participants, however, surprisingly the differences in total energy intake were not significant (1812, 95% CIs [1668, 1956] vs. 1977, 95% CIs [1778, 2175] vs. 2061 kcal/day, 95% CIs [1841, 2281]; p=0.21) (Table 6a). Other noteworthy results were that overweight women consumed a higher percent of dietary fat compared to both normal and obese women (34.0, 95% CIs [32.6, 35.3] vs. 31.3, 95% CIs [30.4, 32.3] vs. 33.9%, 95% CIs [32.4, 35.3]; p=0.001) but less sodium (2691, 95% CIs [2418, 2965] vs. 2726, 95% CIs [2527, 2924] vs. 3019 mg/day, 95% CIs [2716, 3323]; p=0.03).

Other findings were as expected with normal weight women consuming less red meat (1.1, 95% CIs [1.0, 1.3] vs. 1.4, 95% CIs [1.2, 1.6] vs. 1.6 servings/day, 95% CIs [1.4, 1.8]; p=0.0001), more fiber (21.7, 95% CIs [20.5, 22.9] vs. 19.1, 95% CIs [17.5, 20.8] vs. 17.7 g/day, 95% CIs [15.9, 19.5]; p=0.005), vegetables (3.5, 95% CIs [3.2, 3.8] vs. 3.3, 95% CIs [2.9, 3.8] vs. 2.7 servings/day, 95% CIs [2.1, 3.2]; p=0.04), fruits with vegetables (5.2, 95% CIs [4.7, 5.7] vs. 4.9, 95% CIs [4.3, 5.5] vs. 3.8 servings/day, 95% CIs [3.1, 4.5]; p=0.004) than overweight and obese women. Normal weight women were also more physically active than women in the other two categories (9.0, 95% CIs [7.7, 10.3] vs. 7.2, 95% CIs [5.3, 9.0] vs. 5.5 hrs/week, 95% CIs [3.5, 7.5]; p=0.003).

We observed similar results for adherence to WCRF/AICR individual recommendations by BMI status as we noted for ethnicity (Table 6b). The significant difference in dietary intake for meat and sodium and for physical activity corresponded to the health index constituents. A higher proportion of normal weight women, than overweight and obese, met the recommendations for physical activity (72.3 vs. 59.5 vs. 53.3%; p=0.02) and meat (93.6 vs. 85.1 vs. 80.0; p=0.01). The recommendation for sodium intake was not closely followed by any of the weight categories, but obese women were the least likely with only 33.3% adherence followed by normal (52.5%) and overweight (51.4%) (p=0.04). The mean total healthy lifestyle index score, including the BMI component (6.1±1.0 vs. 4.8±1.2 vs. 4.3±1.0; p<.0001) and excluding BMI (5.1±1.0 vs. 4.8±1.2 vs. 4.3±1.0; p<.0001), were both significantly different between BMI categories with normal BMI status having the highest mean score.

Associations with chronic disease biomarkers

Characteristics by low, moderate, and high WCRF/AICR adherers

Moderate associations in biomarker levels were detected between women with low, moderate, and high adherence to WCRF/AICR recommendations (Table 7a). Nonsignificant trends toward significance were observed for urinary E_2, total urinary estrogens, mammographic density, and serum IGF-1. Women who adhered closer to the WCRF/AICR recommendations had higher urinary E_2 (17.0, 95% CIs [14.0, 20.0] vs. 13.9, 95% CIs [12.2, 15.6] vs. 16.1, 95% CIs [13.5, 18.8]; p=0.10), total urinary estrogens (231.7, 95% CIs [195.8, 267.6] vs. 177.2, 95% CIs [156.8, 197.7] vs. 189.1, 95% CIs [157.6, 220.6]; p=0.13), and serum IGF-1 (332.3, 95% CIs [307.9, 356.6] vs.

328.6, 95% CIs [315.1, 342.1] vs. 305.4, 95% CIs [284.8, 325.9]; p=0.11). They also had lower mammographic density (43.3, 95% CIs [36.1, 50.5] vs. 45.3, 95% CIs [41.3, 49.4] vs. 48.4, 95% CIs [42.3, 54.6]; p=0.10). There were not observable associations for serum E_1, serum E_2, urinary E_1, NAF, F2-isoprostane, CRP, and γ-tocopherol.

Characteristics by BMI status

Associations with biomarkers were stronger by BMI status (Table 7b). Mean mammographic density was higher for lower BMI (57.8, 95% CIs [53.6, 61.9] vs. 42.5, 95% CIs [36.7, 48.3] vs. 22.1%, 95% CIs [15.8, 28.4]; p<0.0001). Levels of serum CRP were significantly associated with BMI status (0.9, 95% CIs [0.3, 1.5] vs. 2.3, 95% CIs [1.5, 3.1] vs. 4.6 mg/L, 95% CIs [3.8, 5.5]; p<0.0001) for normal, overweight, and obese individuals respectively. With a higher BMI, we also observed higher mean γ-tocopherol levels (1502, 95% CIs [1298, 1708] vs. 1777, 95% CIs [1488, 2062] vs. 1988 ng/mL, 95% CIs [1690, 2285]; p=0.02). After stratification into low and high γ-tocopherol categories, women were classified as low γ-tocopherol if their γ-tocopherol serum levels were <2500 ng/mL and high if their γ-tocopherol were ≥ 2500 ng/mL, we observed a lower percentage of obese women who were categorized as low γ-tocopherol (68.3%) compared to overweight (86.0%) and normal weight women (86.2%) (p=0.03).

Regression coefficient by total WCRF/AICR score

When determining the estimate and direction of associations using WCRF/AICR recommendations as a continuous variable, we found a significant inverse association with mammographic density (-0.149; p=0.005) (Table 7c). A borderline significant association for serum IGF-1 with WCRF/AICR score was also observed (p=0.07).

Biomarkers and dietary and lifestyle habits

Upon further analysis, we determined regression coefficients between biomarkers and individual dietary intakes (Table 7d). Overall, most biomarkers showed little association with various dietary intakes. The biomarker, which had the strongest association with intake of various dietary constituents, was γ-tocopherol; it was positively associated with meat intake (0.19; p=0.0024) and inversely with fiber (-0.39; p=0.014) and fruits with vegetables (-0.28; p=0.01). Of the different dietary components, alcohol had the most positive significant associations with biomarkers, i.e., serum E_1 (0.018; p=0.03), urinary E_1 (0.028; p=0.03), urinary E_2 (0.029; p=0.01), and urinary F2-isoprostane (0.046; p=0.0018). Whole grain, percent fat, fruit, and physical activity showed no significant association with any biomarker.

Mammographic density and γ-tocopherol levels by total WCRF/AICR score

Figure 1 indicates higher mammographic density with higher WCRF/AICR scores using unadjusted data. This is unlike Table 7a where no association was observed after adjustments for age, parity, ethnicity, luteal phase, and total energy intake. Figure 2 shows that overall, women with high WCRF/AICR scores had lower mean γ-tocopherol levels. Although not significant, data in Table 7a also showed an inverse association with higher adherence to WCRF/AICR scores and γ-tocopherol levels. The low adherence group had a higher mean γ-tocopherol (1844, 95% CIs [1553, 2136] vs. 1641, 95% CIs [1443, 1839] vs. 1590 ng/mL, 95% CIs [1243, 1936]; p=0.57).

Chapter 4: Discussion

Summary

Diet-disease associations are difficult to determine due to the length of time required for quantifiable symptoms to be expressed and chronic diseases to develop. Although disease associated biomarkers can be used for diagnosis, elevated levels in healthy people may also suggest a higher risk for chronic disease development later in life. This study of multiethnic premenopausal women was intended to explore the association between chronic disease biomarkers and a scoring system based on the WCRF/AICR recommendations for cancer prevention using nutritional and lifestyle factors from FFQs.

The results for correlations between 24HRs and FFQs showed that the mean values of dietary intakes were higher in FFQs than 24HRs. For all dietary variables, the correlations between FFQs and 24HRs were moderately strong with the exception of vegetables and fruits with vegetables intake. FFQs lack the ability to include uncommon foods, especially the wide range of vegetables that may be consumed by the women in this study. Also, participants may over report favorable behaviors when responding to 24HRs when interviewed by trained personnel instead of an unmonitored FFQ. Overall, dietary intake for FFQs and 24HRs were similar.

Based on the data for adherence to WCRF/AICR recommendations, we observed that most women met the 8 recommendations operationalized in this study. Healthful behaviors that were closely followed were limiting energy dense food, red meat, and alcohol. Despite the close adherence to most recommendations, we still observed that dietary and lifestyle intakes differed between low, moderate, and high adherers. High

adherers had a low-normal BMI status, consumed less red meat, percent calories from fat, and sodium, while also engaging in more physical activity and intake of fiber and fruits.

We also observed a difference of mean intake by BMI status and ethnic group. Obese women tended to consume more unfavorable foods, e.g., red meat and sodium, and less of the favorable foods, such as vegetables and fiber, as well as being the least physically active. The results corresponded to obese women having lower mean WCRF/AICR scores. Similar patterns were seen for mean WCRF/AICR score by ethnicity, which showed that Native Hawaiians adhered least to the recommendations, mostly driven by higher BMI status, higher red meat and sodium intake, and lower participation in physical activity.

Since the women in this study closely adhered to most recommendations, it may explain why weak associations were detected between adherence to WCRF/AICR recommendations and biomarkers. Higher adherence to recommendations was associated with reduced mammographic density. Borderline associations were also observed for serum γ-tocopherol and IGF-1. Unadjusted data plotted in Figure 1 indicated stronger association with higher WCRF/AICR adherence and reduced serum γ-tocopherol. Overall, this study suggests that the relationship between nutrition and breast cancer risk is weak.

Although only weak associations were detected between biomarkers and WCRF/AICR score, several associations were observed between WCRF/AICR and BMI as well as individual nutrient consumption. With higher BMI status, lower mean mammographic density was observed. However, higher levels of serum CRP and γ-tocopherol were observed with higher BMI. When we stratified γ -tocopherol by low and

high levels, more women with low γ-tocopherol levels were of normal BMI, while women with high γ-tocopherol levels were more likely to be obese.

Of all individual foods, alcohol and red meat consumption were most often associated with biomarker concentrations. The biomarker most strongly associated with diet was γ-tocopherol. Interestingly, mammographic density did not have an association with any individual nutrient, yet it was the only one significantly associated with total WCRF/AICR score.

The WCRF/AICR second expert report documents convincing evidence that alcohol is associated with an increase risk of breast cancer. This agrees with our observations eve with very small intakes of alcohol (4). However, the report also observed probable evidence for body fatness being protective against breast cancer in premenopausal women, but we observed that higher BMI was associated with unfavorable levels of CRP and γ-tocopherol, and poor dietary and lifestyle habits (4).

These findings suggest that dietary and lifestyle behaviors outlined by the WCRF/AICR recommendations for cancer prevention are weakly associated with breast cancer and chronic disease biomarkers. Nonetheless, adherence to WCRF/AICR recommendations still may be protective against chronic disease, especially for at-risk women, such as those who are overweight and obese, by encouraging them to adopt healthful dietary and lifestyle behaviors.

Previous research

To our knowledge this is the first study to examine associations between adherence to WCRF/AICR recommendations and chronic disease biomarkers. However,

previous research investigated adherence to WCRF/AICR recommendations for diet, physical activity, and body fatness with morbidity and mortality in women (69;70). One study observed a 22-24% lower cancer-specific mortality for meeting 1-2 recommendations and 61% for meeting 5-6 recommendations (70). A larger cohort observed similar results with a 34% lower hazard of death for men and women if they met 6-7 recommendations (9).

A previous study that examined a similar nutrition guideline, the Alternative Healthy Eating Index (AHEI), observed an inverse association with another biomarker associated with increased breast cancer, serum E_2. A different study described that lower mammographic densities were associated with dietary patterns of low meat consumption and high intake of plant foods (71).

Mammographic density was inversely associated with higher BMI, which may be the result of increased fat in breast tissue, which reduces the percentage of density. Obesity was also associated with higher CRP and γ-tocopherol levels. These associations may be due to excess adipose tissue or the different dietary patterns associated with BMI status. Obese women consumed more red meat, percent fat, and sodium and these dietary patterns are associated with higher CRP levels (19). Excess adiposity may stimulate inflammatory cytokines which may also influence CRP and γ-tocopherol levels (7;47). Although the type of fat consumed is unknown in the current study, higher uses of plant oils are associated with higher serum γ-tocopherol (72). Surprisingly, there were few significant associations between dietary and lifestyle habits and biomarkers. Of the biomarkers measured, the one most influenced by diet was γ-tocopherol. It was positively associated with meat but inversely associated with fiber, vegetables, and fruits with

vegetables. These findings are contrary to previous studies, which observed an increase of γ-tocopherol levels with plant-oils and plant-based diets (7;72). However, high red meat consumption is associated with inflammatory responses in digestion, which may stimulate an increase in γ-tocopherol (73). Other previous research also observed a rise in γ-tocopherol in response to inflammation especially from type 2 diabetes (74).

Red meat was inversely associated with urinary E_1 and total urinary estrogens, which in turn may indicate higher serum estrogen levels. Previous studies also observed higher urinary estrogens with a vegetarian diet and lower urinary estrogens with an omnivorous diet (27).The positive associations between alcohol intake and biomarkers were similar to previous studies (75). It was interesting that alcohol was positively associated with serum and urinary E_1, total urinary estrogens, and F2-isoprostane despite the low intake of alcohol by our study participants. Although the only significant association between WCRF/AICR recommendations and biomarkers was mammographic density, it was not significantly influenced by any single dietary or lifestyle habit. One study with 1508 premenopausal and postmenopausal women observed lower breast density with a higher intake of saturated fat. For postmenopausal women, the same study also observed a positive association of mammographic density with white wine and an inverse association with red wine (5).

Strengths and limitations

There were many strengths of the present study including the ethnic diversity of the women, as well as the validation of the FFQ to the diverse population of Hawaii. To our knowledge, this is the first analysis studying WCRF/AICR recommendations for

cancer prevention and their associations with biomarkers of chronic disease risk. We used baseline data prior to implementation of the interventions, thereby capturing dietary and lifestyle habits during the past year. The serum, urine, and NAF samples were collected during the luteal phase under standardized conditions and analyzed for biomarkers with highly reproducible results. This is especially important since our study population consisted of premenopausal women. The final strength was the relatively healthy status of the women. Underlying conditions may affect biomarker levels, but in this study we were able to observe associations of typical dietary intakes with biomarkers without confounding through illness.

However, health status was also a limitation of the study. With the healthy status of the women, their high adherence to cancer prevention recommendations, the inclusion criteria to take part in one of the BEAN study, and the location of the study, the participants showed little variability. Therefore, it is difficult to generalize the findings to the general population. The study was also small and with stratification we lost statistical power. Another limitation, as with any dietary or behavior study that uses self-reported data, is the bias to under report unfavorable dietary intake and lifestyle habits and to over report the more favorable behaviors. Although FFQs are generally good to use for large cohort studies because they are cheap and convenient, the number of foods that can be included are limited. Thus, they may miss key intakes of specific populations.

Future research

To expand on this current research, future research could improve the methods by replacing the FFQ dietary assessment with repeated 24HRs, which may provide more

accurate recent dietary habits. They could also use FFQs specially designed to capture foods of interest, e.g. fruit and vegetable intake. Future studies should also recruit a larger group of men and women who vary by adherence to WCRF/AICR recommendations. This may help to detect significant associations. Also, future studies may be interested in studying the associations with smoking because our study focused mostly on the dietary aspect of the recommendations.

Conclusion

Chronic diseases, such as breast cancer, are a growing concern in aging women, but healthful dietary and lifestyle behaviors may decrease disease incidence. Although this study presented weak associations of WCRF/AICR recommendations with chronic disease biomarkers, long-term healthy behaviors are associated with higher life expectancy. Therefore, improved adherence to WCRF/AICR cancer prevention recommendations could substantially reduce incidence and morbidity of chronic disease by encouraging healthy lifestyle and dietary behaviors.

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Table 1. Baseline characteristic of 275 pre-menopausal women from two intervention studies at baseline (n (%) or mean± SD)

Characteristic	BEAN1	BEAN2	BEAN1 & BEAN2
Number of participants	194	81	275
Ethnicity			
White	70 (36.0)	42 (51.8)	112 (40.7)
Asian	77 (39.7)	22 (27.2)	99 (36.0)
Native Hawaiian	24 (12.4)	14 (17.3)	38 (13.8)
Other	23 (11.9)	3 (3.7)	26 (9. 5)
Age	43.0±2.9	39.2±6.1	41.9±4.4
BMI	26.2±5.8	25.9±5.4	26.1±5.7
Height, m	1.6±0.07	1.6±0.06	1.6±0.07
Body weight, kg	68.6±16.9	67.3±14.5	68.2±16.2
Smoking status			
Never	120 (61.8)	59 (72.8)	179 (65.1)
Past	12 (6.2)	5 (6.2)	17 (6.2)
Current	62 (32.0)	17 (21.0)	79 (28.7)
Parity			
Children	145 (74.7)	59 (72.8)	204 (74.2)
No children	49 (25.3)	22 (27.2)	71 (25.8)

Table 2. Correlations for commonly consumed foods assessed by 24HRs and FFQ among 275 pre-menopausal women (mean± SD)[a]

Intakes[b]	**FFQ**	**Recall**	**Correlation coefficient**	***P-value***
Total energy, *kcal/day*	1911±893	1683±379	0.28	*<0.0001*
Red meat, *servings/day*	1.3±1.1	1.2±1.0	0.51	*<0.0001*
Whole grain, *servings/day*	1.8±1.5	1.2±0.9	0.34	*<0.0001*
Fiber, *g/day*	20.1±11.6	16.2±5.4	0.28	*<0.0001*
Percent fat, *%*	32.6±6.0	33.9±5.6	0.46	*<0.0001*
Fruit, *servings/day*	1.5±1.6	1.5±1.0	0.30	*<0.0001*
Vegetable, *servings/day*	3.3±2.4	2.5±1.2	0.05	*0.39*
Fruit & vegetable, *servings/day*	4.8±3.4	4.0±1.7	0.10	*0.094*
Sodium, *mg/day*	2781±1469	2541±764	0.74	*<0.0001*
Alcohol, *drinks/day*	0.3±0.6	0.3±0.5	0.55	*<0.0001*

a. Results obtained from spearman correlations.

b. Intake servings determined by previous food guide pyramid recommendations. Referenced from: Sharma, S., Murphy, S.P., Wilkens, L.R., Au, D., Shen, L., Kolonel, L.N. Extending a multiethnic food composition table to include standardized food group servings. Journal of Food Composition and Analysis, 2003 16:485-495.

Table 3. Scoring for WCRF/AICR cancer prevention recommendations based on data from FFQs[a]

WCRF/AICR Recommendation	Associated recommendations used in this study	Met/did not meet recommendation in this study if:	Score	(N=275) n (%)
1. Body fatness: Be as lean as possible without becoming underweight	Maintain body weight in the normal BMI range	**Met:** 18.5 kg/m^2≤ BMI <25 kg/m^2	1	138 (50.2)
		Did not meet: BMI <18.5 kg/m^2or BMI ≥25 kg/m^2	0	137 (49.8)
2. Physical activity: Be physically active for at least 30 minutes every day	Be moderately or strenuously physically active at least 3.5 hrs per week	**Met**: ≥3.5 *hrs/week*	1	178 (64.7)
		Did not meet: <3.5 *hrs/week*	0	97 (35.3)
3. Energy density: Limit consumption of energy dense foods: avoid sugary drinks	Limit the consumption of sugary drinks to less than 250 g per day	**Met:** <250 *g/day*	1	196 (71.3)
		Did not meet: ≥250 *g/day*	0	79 (28.7)
4. Plant foods: Eat more of a variety of vegetables, fruits, whole grains and legumes such as beans	Eat five servings of fruits and vegetables and 1 serving or more of whole grains	**Met:** ≥5 *servings/day* ≥1 *serving/day*	1	87 (31.6)
		Did not meet: ≥5 *servings/day* <1 *serving/day*	0	18 (6.5)
		Did not meet: <5 *servings/day*	0	170 (61.9)
5. Red meat: Limit consumption of red meats (such as beef, pork and lamb) and avoid processed meats	Limit red meat consumption to less than 2.5 servings per day (2.5 servings based on 500 g per week)	**Met:** <2.5 *servings/day*	1	243 (88.4)
		Did not meet: ≥2.5 *servings/ day*	0	32 (11.6)
6. Alcohol: Limit alcoholic drinks	If alcoholic drinks are consumed, limit consumption to no more than one drink per day	**Met:** <1 *drink/day*	1	248 (90.2)
		Did not meet: ≥1 *drink/day*	0	27 (9.8)
7. Salty foods: Limit consumption of salt; avoid moldy grains or legumes	Limit consumption of salty and processed foods to less than 2400 mg per day	**Met:** <2400 *mg/day*	1	132 (48.0)
		Did not meet: ≥2400 *mg/day*	0	143 (52.0)
8. Smoking: Do not smoke and avoid tobacco smoke	Do not smoke or quit smoking if you are a present smoker	**Met:** Never	1	179 (65.1)
		Met: Past	1	17 (6.2)
		Did not meet: current	0	79 (28.7)
9. Supplements: Aim to meet nutritional needs through diet alone	Dietary supplements are not recommended for cancer prevention	**Not operationalized**	N/A	

a. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical activity, and the Prevention of Cancer: a Global Perspective, Washington, DC: AICR, 2012.

Table 4. Characteristics of women with low, moderate, and high adherence to WCRF/AICR recommendations[a]

	Low n=72	Moderate n=150	High n=53	*p-value*[b]
Age	42.8±3.3	41.5±4.7	41.8±4.9	*0.14*
BMI, *kg/m²*	30.3±6.0	25.5±5.2	22.2±2.0	*<0.0001*
Weight, *kg*	78.3±16.9	66.9±15.6	58.3±7.0	*<0.0001*
Height, *m*	1.6±0.1	1.6±0.1	1.6±0.1	*0.93*
Smoking status				*<0.0001*
Past	7 (41.2)	9(52.9)	1 (5.9)	
Current	35 (44.3)	42 (53.2)	2 (2.5)	
Race				*0.13*
White	23 (20.5)	62 (55.4)	27 (24.1)	
Asian	29 (29.3)	51 (51.5)	19 (19.2)	
Native Hawaiian	12 (31.6)	24 (63.2)	2 (5.2)	
Other	8 (30.8)	13 (50.0)	5 (19.2)	
Data from FFQs[c]				
Total energy, *kcal/day*	2022±846	1900±987	1789±632	*0.34*
Meat, *servings/day*	1.7±1.0	1.2±1.1	0.9±0.7	*<0.0001*
Whole grain, *servings/day*	1.6±1.2	1.9±1.7	2.1±1.5	*0.19*
Fiber, *g/day*	18.1±8.8	20.1±13.1	22.9±10.0	*0.02*
Percent fat, *%*	33.6±6.3	33.0±5.8	30.0±5.6	*0.002*
Fruits, *servings/day*	1.2±1.1	1.5±1.8	1.9±1.3	*0.005*
Vegetables, *servings/day*	2.8±1.8	3.4±2.7	3.6±1.9	*0.12*
Fruits+vegetables, *servings/day*	4.1±2.5	4.9±4.0	5.5±2.5	*0.01*
Alcohol, *drinks/day*	0.5±0.8	0.3±0.5	0.2±0.3	*0.50*
Regular Soda, *g/day*	76.7±117.0	50.4±167.8	16.5±40.4	*0.11*
Sodium, *mg/day*	3171±1364	2811±1622	2163±834	*<0.0001*
Physical activity, *hrs/week*	5.1±8.4	7.4±6.1	12.1±9.9	*<0.0001*

a. Results for continuous variables are means ± standard deviation obtained from proc glm and categorical variables are n (%) obtained from Chi-square, low adherence met < 5 WCRF/AICR recommendations, medium adherence met 5-6 WCRF/AICR recommendations, and high adherence met ≥ 7 WCRF/AICR recommendations.

b. Log-transformed variables were used except age, BMI, weight, height, and percent fat.

c. Intake servings determined by previous food guide pyramid recommendations. Referenced from: Sharma, S., Murphy, S.P., Wilkens, L.R., Au, D., Shen, L., Kolonel, L.N. Extending a multiethnic food composition table to include standardized food group servings. Journal of Food Composition and Analysis, 2003 16:485-495.

Table 5a. Dietary intake and lifestyle factors by ethnic group[a]

	White N=112	**Asian N=99**	**Native Hawaiian N=38**	**Other N=26**	***p-value***[b]
Age[c]	41.8±4.6	42.2±4.3	41.2±4.7	42.2±4.7	*0.70*
BMI, *kg/m*[2d]	26.6	24.5	28.8	26.5	*0.002*
	(25.6-27.6)	(23.4-25.6)	(27.0-30.5)	(24.3-28.6)	
White		0.03	0.16	0.99	
Asian	0.03		0.0005	0.37	
Native Hawaiian	0.16	0.0005		0.37	
Other	0.99	0.37	0.37		
Weight, *kg*	72.2	61.2	74.3	69.0	*<0.0001*
	(69.3-79.1)	(58.2-64.3)	(69.3-79.3)	(63.1-74.9)	
Height, *m*	1.65	1.58	1.62	1.61	*<0.0001*
	(1.63-1.66)	(1.57-1.59)	(1.60-1.64)	(1.59-1.64)	
Smoking status					*0.39*
Never	67 (59.8%)	71 (71.7%)	26 (68.4%)	15 (57.7%)	
Past	8 (7.1%)	7 (7.1%)	1 (2.7%)	1 (3.8%)	
Current	37 (33.1%)	21 (21.2%)	11 (28.9%)	10 (38.5%)	
Data from FFQs[e]					
Total energy,	1934	1926	1905	1760	*0.86*
kcal/day	(1772-2096)	(1751-2100)	(1622-2187)	(1424-2095)	
Red meat,	1.1	1.4	1.6	1.2	*0.0008*
servings/day	(0.9-1.3)	(1.2-1.6)	(1.3-2.0)	(0.8-1.6)	
Whole grain,	2.0	1.8	1.7	1.6	*0.52*
servings/day	(1.7-2.3)	(1.5-2.1)	(1.3-2.2)	(1.1-2.2)	
Fiber,	21.9	18.9	19.4	18.4	*0.03*
g/day	(19.7-24.0)	(16.6-21.2)	(15.7-23.1)	(14.0-22.8)	
Percent fat,	33.0	31.6	33.3	33.2	*0.29*
%	(31.9-34.1)	(30.5-32.8)	(31.4-35.2)	(31.0-35.5)	
Fruit,	1.7	1.4	1.6	1.1	*0.006*
servings/day	(1.5-2.0)	(1.1-1.7)	(1.1-2.1)	(0.5-1.7)	
Vegetable,	3.5	3.1	3.3	2.9	*0.12*
servings/day	(3.1-4.0)	(2.6-3.5)	(2.5-4.0)	(2.0-3.8)	
Fruit & vegetable,	5.3	4.5	4.9	4.0	*0.02*
servings/day	(4.7-5.9)	(3.8-5.1)	(3.8-5.9)	(2.7-5.3)	
Alcohol,	0.5	0.2	0.3	0.3	*0.006*
drinks/day	(0.4-0.6)	(0.1-0.3)	(0.1-0.5)	(0.1-0.5)	
Regular Soda,	57.0	44.7	58.7	40.8	*0.31*
g/day	(30.7-83.2)	(16.4-73.0)	(13.0-104.4)	(18.8-90.0)	
Sodium,	2680	2874	2998	2539	*0.22*
mg/day	(2423-2937)	(2596-3151)	(2550-3446)	(2007-3072)	
Physical activity,	8.9	6.4	6.5	9.5	*0.0008*
hrs/week	(7.4-10.3)	(4.9-8.0)	(4.0-9.1)	(6.5-12.5)	

a. Results for continuous variables are least-square means and confidence intervals, obtained from proc glm and adjusted for age and BMI, Results for categorical variables are n (%), obtained from Chi-square test.

b. Log-transformed variables were used except categorical variables, age, BMI, weight, height, and percent fat.

c. Result for Age are means ± standard deviation, obtained from proc glm, unadjusted.

d. P-values from Tukey test between ethnic groups are given to determine which significantly differed from each other.

e. Intake servings determined by previous food guide pyramid recommendations. Referenced from: Sharma, S., Murphy, S.P., Wilkens, L.R., Au, D., Shen, L., Kolonel, L.N. Extending a multiethnic food composition table to include standardized food group servings. Journal of Food Composition and Analysis, 2003 16:485-495.

Table 5b. Individual WCRF/AICR constituents and mean WCRF/AICR score by ethnic group[a]

	White N=112	**Asian N=99**	**Native Hawaiian N=38**	**Other N=26**	***p-value***
	Met (%)	Met (%)	Met (%)	Met (%)	
BMI	56 (50.0)	61 (61.6)	10 (26.3)	11 (42.3)	*0.002*
Physical activity[b]	88 (78.6)	52 (52.5)	21 (55.3)	17 (65.4)	*0.0006*
White		0.0005	0.03	0.58	
Asian	0.0005		0.99	0.59	
Native Hawaiian	0.03	0.99		0.80	
Other	0.58	0.59	0.80		
Alcohol	100 (89.3)	90 (90.9)	34 (89.5)	24 (92.3)	*0.96*
Fruit & vegetable	43 (38.4)	26 (26.3)	11 (29.0)	7 (26.9)	*0.25*
Meat	104 (92.9)	87 (87.9)	30 (78.9)	22 (84.6)	*0.12*
Soda	103 (92.0)	92 (92.9)	32 (84.2)	24 (92.3)	*0.42*
Smoke	75 (67.0)	78 (78.8)	27 (71.0)	16 (61.5)	*0.18*
Sodium	57 (50.9)	46 (46.5)	16 (42.1)	13 (50.0)	*0.79*
Mean score[b]	5.6±1.3	5.4±1.3	4.8±1.2	5.1±1.3	*0.007*
White		0.65	0.003	0.42	
Asian			0.05	0.88	
Native Hawaiian				0.57	

a. Results for categorical variables are n (%), obtained from Chi-square test, results for continuous variables are means ± standard deviation, obtained from proc glm.

b. P-values from Tukey test between ethnic groups are given to determine which significantly differed from each other.

Table 6a. Dietary intake and lifestyle factors by BMI status[a]

	Normal n=141	Overweight n=74	Obese n=60	*p-value*[b]
Age	41.5	42.6	41.9	*0.22*
BMI, *kg/m²*	22.0 (21.5-22.4)	27.0 (26.4-27.6)	34.9 (34.2-35.5)	*<0.0001*
Weight, *kg*	57.0 (55.6-58.5)	70.8 (68.6-72.8)	91.7 (89.4-94.0)	*<0.0001*
Height, *m*	1.6 (1.6-1.6)	1.6 (1.6-1.6)	1.6 (1.6-1.6)	*0.34*
Smoking Status				*0.12*
Never	96 (53.6%)	48 (26.8%)	35 (19.6%)	
Past	9 (52.9%)	7 (41.2%)	1 (5.9%)	
Current	36 (45.6%)	19 (24.0%)	24 (30.4%)	
Data from FFQs[c]				
Total energy, *Kcal/day*	1812 (1668-1956)	1977 (1778-2175)	2061 (1841-2281)	*0.21*
Red meat, *servings/day*	1.1 (1.0-1.3)	1.4 (1.2-1.6)	1.6 (1.4-1.8)	*<0.0001*
Whole grains, *servings/day*	2.0 (1.8-2.2)	1.7 (1.5-2.0)	1.6 (1.3-2.0)	*0.17*
Fiber, *g/day*	21.7 (20.5-22.9)	19.1 (17.5-20.8)	17.7 (15.9-19.5)	*0.005*
Percent fat, *%*	31.3 (30.4-32.3)	34.0 (32.6-35.3)	33.9 (32.4-35.3)	*0.001*
Fruit, *servings/day*	1.7 (1.5-1.9)	1.6 (1.3-1.9)	1.1 (0.8-1.5)	*0.09*
Vegetable, *servings/day*	3.5 (3.2-3.8)	3.3 (2.9-3.8)	2.7 (2.1-3.2)	*0.04*
Fruit & vegetables, *servings/day*	5.2 (4.7-5.7)	4.9 (4.3-5.5)	3.8 (3.1-4.5)	*0.004*
Alcohol, *drinks/day*	0.4 (0.3-0.5)	0.4 (0.2-0.5)	0.2 (0.1-0.4)	*0.12*
Regular Soda, *g/day*	60.6 (37.8-83.4)	28.1 (-3.3-59.5)	55.6 (20.1-90.4)	*0.11*
Sodium, *mg/day*	2726 (2527-2924)	2691 (2418-2965)	3019 (2716-3323)	*0.03*
Physical activity, *hrs/week*	9.0 (7.7-10.3)	7.2 (5.3-9.0)	5.5 (3.5-7.5)	*0.003*

a. Results for continuous variables are least-square means and confidence intervals, obtained from proc glm and adjusted for age and energy, Results for categorical variables are n (%), obtained from Chi-square test. BMI status: Normal <24.9 *kg/m²*, Overweight =25-29.9 *kg/m²*, Obese >30 *kg/m²*.

b. Log-transformed variables were used except categorical variables, age, BMI, weight, height, and percent fat.

c. Intake servings determined by previous food guide pyramid recommendations. Referenced from: Sharma, S., Murphy, S.P., Wilkens, L.R., Au, D., Shen, L., Kolonel, L.N. Extending a multiethnic food composition table to include standardized food group servings. Journal of Food Composition and Analysis, 2003 16:485-495.

Table 6b. Individual WCRF/AICR constituents and mean WCRF/AICR score by BMI status[a]

	Normal n=141	**Overweight n=74**	**Obese n=60**	***p-value***
	Met (%)	Met (%)	Met (%)	
Physical activity	102 (72.3)	44 (59.5)	32 (53.3)	*0.02*
Alcohol	128 (90.8)	63 (85.1)	57 (95.0)	*0.15*
Fruit & vegetable	50 (35.5)	23 (31.1)	14 (23.3)	*0.24*
Meat	132 (93.6)	63 (85.1)	48 (80.0)	*0.01*
Soda	129 (91.5)	70 (94.6)	52 (68.7)	*0.27*
Smoking	105 (74.5)	44 (74.3)	36 (60.0)	*0.09*
Sodium	74 (52.5)	38 (51.4)	20 (33.3)	*0.04*
Mean score	6.1±1.0	4.8±1.2	4.3±1.0	*<0.0001*
Mean score[b]	5.1±1.0	4.8±1.2	4.3±1.0	*<0.0001*

a. Results for categorical variables are n (%), obtained from Chi-square test, results for continuous variables are means ± standard deviation, obtained from proc glm. BMI status: Normal <24.9 kg/m^2, Overweight =25-29.9 kg/m^2, Obese >30 kg/m^2.
b. We removed the BMI individual score from the mean score.

Table 7a. Biomarker levels of women with low, moderate, and high adherence to WCRF/AICR recommendations[a]

	Low adherence n=72	**Moderate adherence n=150**	**High adherence n=53**	***p-value***[b]
Serum E_1, *pg/mL*[c]	96.8 (83.2-110.4)	96.1 (87.5-104.6)	100.7 (85.5 -115.9)	*0.78*
Serum E_2, *pg/mL*[c]	151.9 (130.7-173.0)	142.8 (129.5-156.1)	149.7 (126.0-173.3)	*0.47*
Urinary E_1 molar, *pmol/mg*[c]	40.2 (32.9-47.4)	39.7 (35.0-44.4)	49.2 (40.9-57.4)	*0.42*
Urinary E_2 molar, *pmol/mg*[c]	16.1 (13.5-18.7)	13.9 (12.2-15.6)	17.0 (14.0-20.0)	*0.10*
Total urinary estrogens, *pmol/mg*[c]	189.1 (157.6-220.6)	177.2 (156.8-197.7)	231.7 (195.8-267.6)	*0.13*
Nipple aspirate fluid, *µL*[d]	34.3 (13.5-55.1)	34.6 (23.1-46.0)	42.5 (22.3-62.8)	*0.39*
Urinary F2-isoprostane, *ng/mL*[d]	16.4 (13.4-19.4)	14.5 (12.8-16.1)	12.9 (10.0-15.8)	*0.45*
Mammographic density, *%*[c]	48.4 (42.3-54.6)	45.3 (41.3-49.4)	43.3 (36.1-50.5)	*0.10*
Serum IGF-1, *ng/mL*[e]	305.4 (284.8-325.9)	328.6 (315.1-342.1)	332.3 (307.9-356.6)	*0.11*
Serum C-reactive protein, *mg/L*[e]	1.5 (0.7-2.3)	2.2 (1.7-2.7)	2.8 (1.9-3.7)	*0.96*
Serum **γ**-tocopherols, *ng/mL*[e,f]	1844 (1553-2136)	1641 (1443-1839)	1590 (1243-1936)	*0.57*
Low **γ**-tocopherols	36 (75.0%)	75 (83.3%)	29 (87.9%)	0.30
High **γ**-tocopherols	12 (25.0%)	15 (16.7%)	4 (12.1%)	

a. Results for continuous variables are least-square means and confidence intervals, obtained from proc glm and adjusted for age, parity, luteal phase, ethnicity, total energy intake, and BMI. Results for categorical variables are n (%) obtained from Chi-square, low adherence met 5, moderate adherence 5-6, high adherence met ≥ 7 WCRF/AICR recommendations.

b. Log-transformed variables were used.

c. Samples collected in both BEAN studies.

d. Samples collected in BEAN2 study only.

e. Samples collected in BEAN1 study only.

f. Low γ-tocopherol <2500 *ng/ml*, High γ-tocopherol ≥2500 *ng/ml*, based from: Cooney, R.V., Franke, A.A., Wilkens, L.R., Gill, J., Kolonel, L.N. Elevated plasma γ-tocopherol and decreased alpha-tocopherol in men are associated with inflammatory markers and decreased plasma 25-OH Vitamin D. Nutrition and Cancer, 2008, 60:S1, 21-29.

Table 7b. Biomarker levels by BMI status[a]

	Normal n=141	**Overweight n=74**	**Obese n=60**	***p-value*[b]**
Serum E_1, *pg/mL*[c]	97.8 (88.9-106.7)	95.2 (83.0-107.3)	98.2 (84.6-111.8)	*0.84*
Serum E_2, *pg/mL*[c]	156.4 (142.5-170.2)	138.8 (120.0-157.7)	132.9 (111.9-154.0)	*0.17*
Urinary E_1 molar, *pmol/mg*[c]	42.9 (38.1-47.8)	37.4 (30.7-44.2)	43.8 (36.4-51.1)	*0.52*
Urinary E_2 molar, *pmol/mg*[c]	14.5 (12.7-16.2)	14.6 (12.1-17.0)	17.0 (14.4-19.7)	*0.19*
Total urinary E molar, *pmol/mg*[c]	199.8 (178.5-221.0)	171.8 (142.2-201.4)	193.5 (161.5-225.6)	*0.64*
Nipple aspirate fluid, *µL*[d]	38.1 (26.1-50.1)	41.9 (26.9-57.0)	21.3 (1.0-41.5)	*0.60*
Urinary F2-isoprostane, *ng/mL*[d]	14.4 (12.7-16.1)	13.3 (11.1-15.4)	17.3 (14.4-20.2)	*0.06*
Mammographic density, *%*[e]	57.8 (53.6-61.9)	42.5 (36.7-48.3)	22.1 (15.8-28.4)	*<0.0001*
Serum IGF-1, *ng/mL*[e]	325.2 (311.2-339.1)	334.3 (314.4-354.2)	305.5 (284.7-326.3)	*0.13*
Serum C-reactive protein, *mg/L*[e]	0.9 (0.3-1.5)	2.3 (1.5-3.1)	4.6 (3.8-5.5)	*<0.0001*
Serum **γ**-tocopherols, *ng/mL*[e,f]	1502 (1298-1708)	1777 (1488-2062)	1988 (1690-2285)	*0.02*
Low **γ**-tocopherols	75 (86.2%)	37 (86.0%)	28 (68.3%)	0.03
High **γ**-tocopherols	12 (13.8%)	6 (14.0%)	13 (31.7%)	

a. Results for continuous variables are least-square means and confidence intervals, obtained from proc glm and adjusted for age, parity, luteal phase, ethnicity, and total energy intake, results for categorical variables are n (%) obtained from Chi-square, BMI status: Normal <24.9 *kg/m*^{2}, Overweight =25-29.9 *kg/m*^{2}, Obese >30 *kg/m*^{2}.

b. Log-transformed variables were used.

c. Samples collected in both BEAN studies.

d. Samples collected in BEAN2 study only.

e. Samples collected in BEAN1 study only.

f. Low γ-tocopherol <2500 *ng/ml*, High γ-tocopherol ≥2500 *ng/ml*, based from: Cooney, R.V., Franke, A.A., Wilkens, L.R., Gill, J., Kolonel, L.N. Elevated plasma γ-tocopherol and decreased alpha-tocopherol in men are associated with inflammatory markers and decreased plasma 25-OH Vitamin D. Nutrition and Cancer, 2008, 60:S1, 21-29.

Table 7c. Association of biomarkers with adherence to WCRF/AICR recommendations[a]

	Estimate	***p-value***[b]
Serum E_1, *pg/mL*[c]	-0.007	*0.79*
Serum E_2, *pg/mL*[c]	-0.009	*0.75*
Urinary E_1 molar, *pmol/mg*[c]	0.03	*0.36*
Urinary E_2 molar, *pmol/mg*[c]	-0.004	*0.91*
Total urinary E molar, *pmol/mg*[c]	0.03	*0.30*
Nipple aspirate fluid, *μL*[d]	0.08	*0.40*
Urinary F2-isoprostane, *ng/mL*[d]	-0.04	*0.27*
Mammographic density, *%*[e]	-0.15	*0.005*
Serum IGF-1, *ng/mL*[e]	0.02	*0.071*
Serum C-reactive protein, *mg/L*[e]	0.01	*0.88*
Serum **γ**-tocopherols, *ng/mL*[e]	-0.06	*0.19*

a.. Results are estimates, obtained from proc glm and adjusted for age, parity, luteal phase, ethnicity, BMI, and total energy intake.
b. Log-transformed variables were used.
c. Samples collected in both BEAN studies.
d. Samples collected in BEAN2 study only.
e. Samples collected in BEAN1 study only.

Table 7d. Association of food group and physical activity with breast cancer biomarkers[a]

	BEAN1 & BEAN2[b] n=275					BEAN1[c] n=194		BEAN2[d] n=81
	Serum E_1, *pg/mL*	**Serum E_2, *pg/mL***	**Urinary E_1, *pmol/mg***	**Urinary E_2, *pmol/mg***	**Total urinary estrogens, *pmol/mg***	**Mammographic *density, %***	**IGF-1, *ng/mL***	**NAF, *µL***
Total Energy	-0.01	-0.007	0.10	0.20	0.08	0.12	0.008	0.14
p-value	*0.82*	*0.92*	*0.29*	*0.02*	*0.35*	*0.45*	*0.84*	*0.50*
Meat	-0.01	-0.04	-0.13	-0.07	-0.15	-0.25	0.04	-0.005
p-value	*0.71*	*0.34*	*0.01*	*0.17*	*0.002*	*0.75*	*0.07*	*0.97*
Whole grains	-0.07	-0.04	-0.03	-0.06	0.004	-0.04	0.03	0.06
p-value	*0.08*	*0.38*	*0.62*	*0.26*	*0.94*	*0.67*	*0.24*	*0.65*
Fiber	0.002	-0.03	0.01	-0.13	0.06	0.10	0.09	-0.54
p-value	*0.93*	*0.75*	*0.91*	*0.24*	*0.60*	*0.59*	*0.05*	*0.09*
Percent fat	-0.0006	-0.003	-0.005	-0.004	-0.001	0.002	0.00007	0.02
p-value	*0.90*	*0.63*	*0.48*	*0.51*	*0.82*	*0.82*	*0.98*	*0.42*
Fruit	-0.05	-0.04	0.001	0.02	0.007	0.03	0.01	-0.07
p-value	*0.08*	*0.23*	*0.97*	*0.60*	*0.85*	*0.64*	*0.47*	*0.58*
Vegetable	0.09	-0.01	0.09	-0.02	0.06	0.12	0.03	-0.39
p-value	*0.12*	*0.87*	*0.28*	*0.74*	*0.42*	*0.37*	*0.45*	*0.93*
Fruit & vegetable	0.04	-0.03	0.03	-0.06	0.005	0.15	0.03	-0.41
p-value	*0.52*	*0.58*	*0.71*	*0.41*	*0.94*	*0.26*	*0.36*	*0.06*
Alcohol	0.02	0.01	0.03	0.03	0.02	0.02	-0.005	0.04
p-value	*0.03*	*0.25*	*0.03*	*0.01*	*0.12*	*0.25*	*0.23*	*0.33*
Soda	1.03	0.03	0.03	0.03	0.02	-0.01	-0.002	0.02
p-value	*0.50*	*0.03*	*0.11*	*0.052*	*0.17*	*0.65*	*0.82*	*0.55*
Sodium	0.21	0.13	0.04	-0.01	-0.02	0.33	-0.12	-0.74
p-value	*0.004*	*0.13*	*0.73*	*0.92*	*0.81*	*0.09*	*0.02*	*0.07*
Physical activity	0.003	-0.0005	-0.02	-0.03	-0.01	-0.08	-0.02	0.12
p-value	*0.91*	*0.99*	*0.57*	*0.46*	*0.78*	*0.23*	*0.24*	*0.23*

a. Results are estimates, obtained from proc glm and adjusted for age, parity, luteal phase, ethnicity, BMI, and total energy intake from log-transformed variables.

b. Samples collected in both BEAN studies.

c. Samples collected in BEAN1 study only.

d. Samples collected in BEAN2 study only.

Table 7e. Association of food group and physical activity with inflammatory biomarkers[a]

	BEAN1[b] n=194		BEAN2[c] n=81
	CRP, *mg/L*	**γ-tocopherol, *ng/mL***	**F2-isoprostane, *ng/mL***
Total energy	0.01	0.11	-0.05
p-value	*0.96*	*0.42*	*0.58*
Meat	0.08	0.19	0.001
p-value	*0.46*	*0.002*	*0.99*
Whole grains	-0.16	-0.13	-0.006
p-value	*0.25*	*0.080*	*0.92*
Fiber	-0.31	-0.39	0.06
p-value	*0.27*	*0.01*	*0.66*
Percent fat	0.006	0.002	-0.001
p-value	*0.72*	*0.81*	*0.90*
Fruit	-0.13	-0.09	0.03
p-value	*0.15*	*0.06*	*0.59*
Vegetable	0.001	-0.28	0.007
p-value	*0.99*	*0.01*	*0.93*
Fruit & vegetable	-0.12	-0.28	0.04
p-value	*0.52*	*0.01*	*0.66*
Alcohol	-0.03	0.003	0.05
p-value	*0.27*	*0.86*	*0.002*
Soda	-0.01	0.02	-0.03
p-value	*0.81*	*0.40*	*0.03*
Sodium	-0.34	0.06	0.07
p-value	*0.23*	*0.71*	*0.69*
Physical activity	0.07	0.03	0.04
p-value	*0.48*	*0.61*	*0.34*

a. Results are estimates, obtained from proc glm and adjusted for age, parity, luteal phase, ethnicity, BMI, and total energy intake from log-transformed variables.
b. Samples collected in BEAN1 study only.
c. Samples collected in BEAN2 study only.

Figure 1. Mammographic density percent by WCRF/AICR score for 194 women

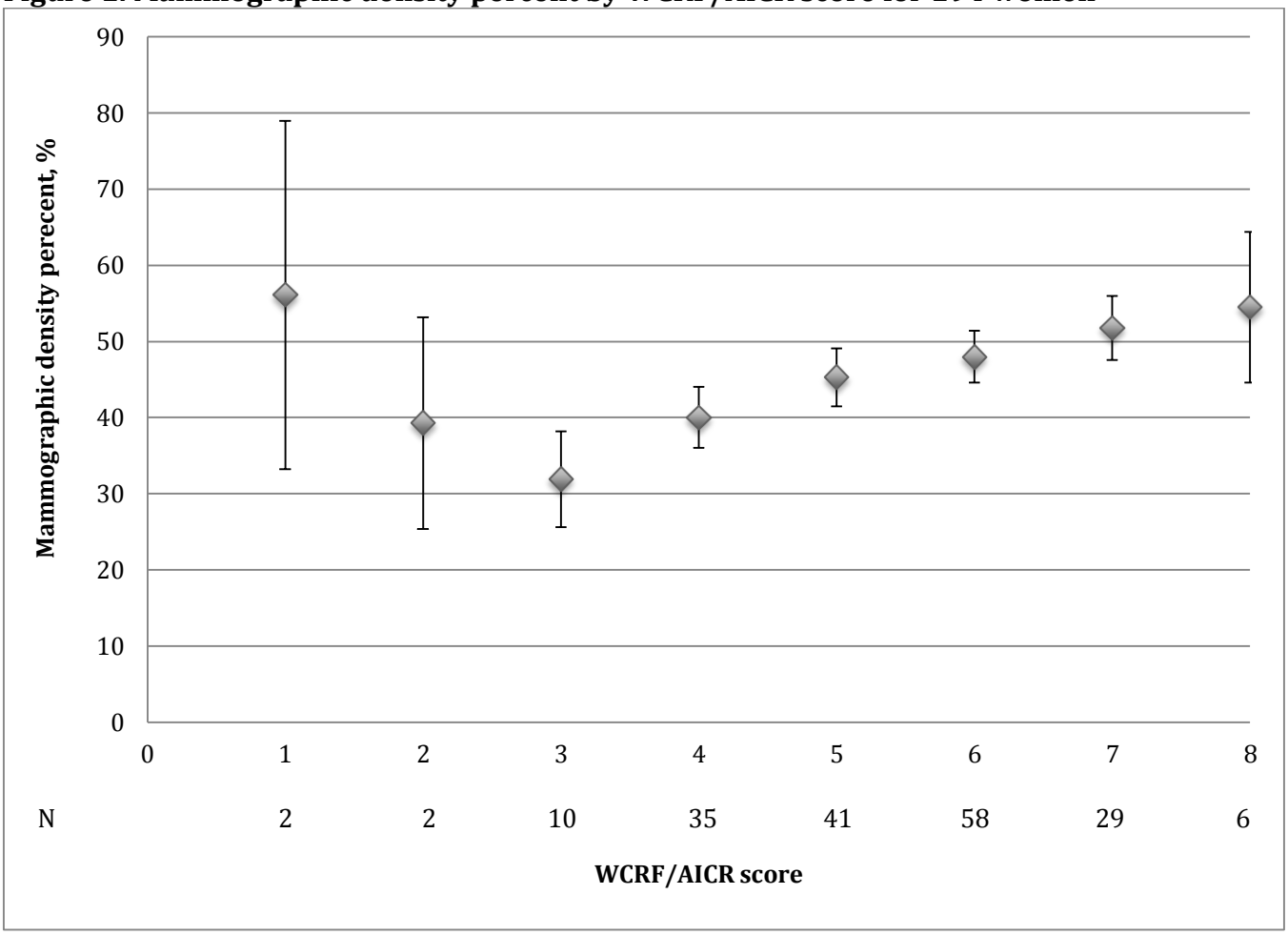

Figure 2. Serum γ-tocopherol by WCRF/AICR score in 194 women