DIETARY INTAKE OF PHYTOESTROGENS AND ESTROGEN RECEPTOR STATUS IN PREMENOPAUSAL BREAST CANCER PATIENTS

A THESIS

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To the Dean of Graduate Studies and Research:

I am submitting herewith a thesis written by Marina S. Touillaud entitled "Dietary Intake of Phytoestrogens and Estrogen Receptor Status in Premenopausal Breast Cancer Patients". I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Nutrition.

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ABSTRACT

Dietary Intake of Phytoestrogens and Estrogen Receptor Status in Premenopausal Breast Cancer Patients

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While diet may influence estrogen receptor (ER) status, no study has investigated the effect of phytoestrogen intake on this breast cancer prognostic factor. This case-case study examined ER status and usual pre-diagnostic phytoestrogen consumption of 93 premenopausal breast cancer patients. Data were collected from a 207-item food frequency questionnaire and abstracted from medical records. ER-status groups were similar in demographic and anthropometric characteristics, oral contraceptive use, and caloric intake. In univariate analyses, a low intake of genistein, the most potent isoflavone, was associated with increased risk of ER-negative compared to ER-positive breast cancer [odds ratio (OR) = 1.76, 95% confidence interval (CI) = 0.73 – 4.25], while the relationship was weaker for total isoflavones (OR = 1.03, 95% CI = 0.43 – 2.46). Low total phytosterol intake was associated with decreased risk of ER-negative cancer (OR = 0.57, 95% CI = 0.24 – 1.37). These findings suggest that phytoestrogens may influence ER status.

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CHAPTER 1

Introduction

Breast cancer is the most common malignancy among women in the United States and accounts for 30% of female cancers. According to the American Cancer Society, 192,200 new cases of breast cancer will be diagnosed in 2001 (Greenlee, Hill-Harmon, Murray, & Thun, 2001). As the second highest cause of cancer death after lung cancer among American women overall, breast cancer remains the leading cause of death among women between the ages of 40 and 59 years (Greenlee, Murray, Bolden, & Wingo, 2000). However, in the last 10 years, age-adjusted breast cancer mortality rates have declined steadily despite incidence rates remaining relatively level. We can speculate that the encouraging trend in mortality figures is due to the earlier detection of disease as a result of screening programs, as well as advances in multimodal therapies (Ries et al., 2000).

Among host factors that influence the etiology and progression of breast cancer tumors, the estrogen environment is considered to play a major role. Estrogens are steroid hormones derived from cholesterol, and include 17β-estradiol, estrone, and estriol. They are primarily secreted by the ovaries in premenopausal women and by adipose tissue in postmenopausal women. Their effect in normal breast tissue is to induce the expression of genes coding for a large number of enzymes and growth factors involved in breast cell growth. Still active in malignant breast cells, estrogens

participate in tumor growth promotion and later in progression of the disease (Bashirelahi, Koffman, & Sydiskis, 1997).

Estrogens exert their proliferative action on target tissues through the binding and activation of nuclear estrogen receptors (ERs) that induce a sequence of genomic and cellular responses. In normal breast tissue cells, estrogens regulate the expression of their own receptors through a feedback mechanism. ERs are involved in determining the ultimate response of an individual cell to estrogenic stimulation. Indeed, high levels of these high-affinity steroid receptors are found particularly in estrogen-sensitive tissues such as breast. In medical practice, to assess tissue sensitivity of breast tumors to estrogens, cellular levels of ERs are measured by immunocytochemical assays in surgically-removed malignant tissue and are used to define the ER status of a tumor. A breast cancer cell is ER-positive if cellular ER level is greater than or equal to 10 femtomoles (10^{-15}) of receptor per milligram cytosol protein (fmol/mg). The overall tumor is considered ER-positive if more than 10% cells are ER-positive.

In the United States, about two thirds of newly-diagnosed breast cancer cases are ER-positive (Hortobagyi & Dhingra, 1993). Clinical studies have shown that ER-positive tumors tend to be more differentiated and less aggressive, to respond more favorably to endocrine therapy, and to be associated with better prognosis compared to ER-negative tumors. Overall, ER status of tumors correlates with a number of established prognostic biomarkers, and its measurement has become a standard practice in the management of breast cancer.

While a large number of studies have focused on identifying breast cancer risk factors, little research has been conducted specifically on the determinants of ER status despite the clinical importance of ERs in breast cancer treatment. To date, only nine studies altogether have specifically examined the relationship between ER status and any dietary factors. In general, these studies have described positive associations between ER-positive status of breast tumors and the consumption of vegetables, as well as vitamin and fiber intake (Ingram, Roberts, & Nottage, 1992; Rock, Saxe, Ruffin, August, & Schottenfeld, 1996). These studies suggest that compounds found in vegetables and fruits may be potential factors influencing ER status.

Epidemiologic research on breast cancer in which particular attention is paid to plant foods is further supported by strong evidence for the protective effects of fruits and vegetables against the development of other types of cancer. Consumption of phytoestrogens, plant components that have weak estrogenic effects, has been a recent focus in attempting to explain some of the large international and geographical differences in breast cancer incidence. Primarily, ecological and international studies have suggested a protective role of phytoestrogens in breast cancer given the low breast cancer mortality rates in Asia where soy-based foods, products which are rich in phytoestrogens, are commonly consumed (Adlercreutz et al., 2000). Further evidence comes from a few case-control studies and from some migrant studies showing a substantial impact of Western diets, which are low in phytoestrogens, on breast cancer risk. Animal and cellular studies of cancer have reported the inhibiting action of

phytoestrogens on the development of cancer (Messina, Persky, Setchell, & Barnes, 1994). Thus, epidemiologic, *in vivo*, and laboratory studies support the protective effects of phytoestrogens against the development of cancer.

Phytoestrogens are found in fruits and vegetables but are most prevalent in soybeans, other legumes, whole-grain foods, nuts, and various seeds (Pillow et al., 1999). Structurally similar to endogenous estrogens, phytoestrogens are classified into subgroups including isoflavones, flavones, flavonols, phytosterols, coumestans, and lignans. *In vitro*, animal and epidemiologic studies show a variety of phytoestrogen effects, including antioxidative, antiproliferative, estrogenic, and antiestrogenic, in a wide range of cancers (Messina, 1999). Dietary estrogens mimic the action of endogenous estrogens by binding to ERs and providing many of the biologic effects observed with endogenous estrogens. Conversely, phytoestrogens have been shown to exhibit antagonist effects against estrogen, much like selective estrogen receptor modulators (SERMs), important agents in the prevention and treatment of breast cancer and osteoporosis, that are competitive inhibitors of estrogens functioning as either estrogenic or antiestrogenic (Dutertre & Smith, 2000). Finally, given their possible anticarcinogenic effects, phytoestrogens have been postulated as candidates in preventing initiation or slowing the growth of estrogen-sensitive types of cancer.

In summary, there is much evidence for significant associations between selected nutritional factors and ER status, between ER status and prognosis of breast cancer, and also between nutritional factors and breast cancer survival. To date, no published studies

on the association between dietary factors and ER status in breast cancer have addressed phytoestrogen intake. Using a unique nutritional database, the present study examined the effects of dietary intake of phytoestrogens in the usual prediagnostic diet of premenopausal women with breast cancer on ER status. This study used a case-case design, similar to a case-comparison design where the comparison group is also comprised of breast cancer patients. The ultimate issue of this research is to identify the effects of dietary phytoestrogens on biologic characteristics of cancer and to identify the mechanisms by which dietary factors may influence breast cancer survival. I have been involved in all aspects of this study, including the development of the research question, medical record abstraction, food frequency questionnaire processing, and have been responsible for conducting the nutritional analyses.

Statement of the Problem

The research hypotheses of this study were that premenopausal women diagnosed with breast cancer who had in their usual prediagnostic diet high dietary intake (i.e., greater than the sample median) of: a) genistein; b) total isoflavones; and c) total phytosterols would have been more likely to be diagnosed with ER-positive tumors than those who consumed lower amounts of these phytoestrogens.

Null hypothesis

Compared with premenopausal women diagnosed with ER-negative breast cancer tumors, women diagnosed with ER-positive breast cancer tumors had the same level of:

a) genistein intake; b) total isoflavone intake; and c) total phytosterol intake in their usual prediagnostic diet.

CHAPTER 2

Review of the literature

Definition and biological roles of ERs

There are two subtypes of ERs coded by two separate genes. The first form, ERα, was identified in 1969 and cloned in 1985, and the second form, ERβ, was discovered recently in 1996 (Krishnan, Heath, & Bryant, 2000). ERα and ERβ are differentially distributed in the body and within tissues. Although ERβ appears to be the more generally expressed form, ERα is predominantly detected in the breast tissue.

The primary role of ERs is to bind to estrogens and other steroid receptor coactivators that then interact with a genomic response element, inducing the transcriptional activation of estrogen-responsive genes. ERα and ERβ show partial homology between their ligand-binding domains, but differ in their mode of regulation and appear clearly to have distinct biologic roles. While ERα has a dominant role in the female reproductive tract including the breast tissue, ERβ might have a protective role against the mitogenic activity of estrogens in breast cell proliferation and carcinogenesis (Gustafsson, 1999; Roger et al., 2001).

Mechanisms of ER expression in breast cancer

As breast cancer progression is often associated with the development of estrogen resistance, the loss of functional ERs in ER-negative tumors has been investigated for its

role in breast carcinogenesis. Several types of genetic mutations or deletions that involve the ER gene are known to relate to estrogen resistance in breast cancer. Alternative causes of estrogen resistance suggest alteration in the estrogen response pathway or alteration in ER gene expression (Bashirelahi et al., 1997).

To explain the different biological and clinical attributes between tumors, three main hypotheses have been raised about the relationship between ER-positive and ER-negative breast cancers (Zhu, Bernard, Levine, & Williams, 1997). One hypothesis considers the two types of tumors as different entities that have different etiologic pathways and factors, while another considers ER status as an indicator of different stages in disease progression resulting from clonal evolution. However, research does not consistently support these theories. Zhu et al. (1997) suggest a third possibility: according to these authors, ER status may result from the effects of various factors after tumor initiation, including exogenous factors such as life-style or diet. Regardless of the role ERs play in breast carcinogenesis, research to identify factors that may mediate ER status at diagnosis is relevant for the ultimate development of prevention strategies against ER-negative breast cancers.

Determinants of ER status in breast cancer

To identify modifiable factors for ER status, the research has primarily investigated factors previously described as being related to breast cancer risk. Relationships between ER status and various life-style factors, including smoking, geographic area, breast-feeding, and the number of mammograms in the past five years, have been

described in the literature (Jain & Miller, 1997; Nasca et al., 1994; Rock et al., 1996). Several patient characteristics such as the breast cancer genes, age, menopausal status, exogenous estrogen use, and body weight are other factors associated with ER status (Kushi et al., 1995; Rock et al., 1996). Although a causal relationship is difficult to establish between ER status and life-style and biologic factors, these factors are important to consider at least as markers of ER status in the management of breast cancer.

Through an extensive search in Medline, nine studies evaluating dietary factors in association with ER status in breast cancer were found. Compared to ER-negative breast cancer patients, women with ER-positive tumors are more likely to have a higher consumption of green and yellow vegetables, fiber, grain cereals, as well as a higher intake of total vitamin A, carotenoids and vitamin B6 (Ingram et al., 1992; Rock et al., 1996). There are conflicting findings on the effects of dietary fat and carbohydrates on ER status (Harlan et al., 1993; Hislop, Kan, Coldman, Band, & Brauer, 1988; Holm et al., 1989; Jain & Miller, 1997; Kushi et al., 1995; Verreault et al., 1988). There is no clear evidence of a relationship between ER status and alcohol consumption (Nasca et al., 1994). Altogether, these studies suggest that, rather than the total energy intake or the source of energy (fat or carbohydrates), specific nutrients may be potential factors for ER status, including saturated fat, cholesterol, and plant micronutrients.

Epidemiology of phytoestrogens in breast cancer

Human studies suggesting a protective effect of phytoestrogens against breast cancer have mostly addressed the risk of developing the disease rather than ER status. Several ecological studies and migrants studies of Japanese population to the United States have suggested a protective effect of the consumption of soy-based foods, rich in isoflavones and particularly genistein, against breast cancer, as reviewed by Adlercreutz et al. (2000) and McMichael-Phillips et al. (1998). Laboratory studies showing the growth-inhibitory effect of phytoestrogens derived from soy in cell culture systems and animal models of breast cancer support these findings (Kurzer & Xu, 1997; McMichael-Phillips et al., 1998).

A few case-control trials were performed concerning the association between phytoestrogens and breast cancer risk. An epidemiologic study carried out in Singapore found an inverse association between the consumption of soybean products and breast cancer risk in premenopausal women (Lee et al., 1991). In contrast, one study in China failed to find a similar relationship (Yuan, Wang, Ross, Henderson, & Yu, 1995). Although overall the case-control studies are conflicting, they provide at least a weak evidence for the protective effects of isoflavone-rich diets against the risk of breast cancer (McMichael-Phillips et al., 1998; Wu, 2000). Further data suggest that exposure to phytoestrogens before birth or during adolescence may also decrease risk of breast cancer in women, supporting the hypothesis of the antiestrogenic effects of isoflavones in a high-estrogen environment (Adlercreutz et al., 2000; Shu et al., 2001). All these

studies of phytoestrogens were based on soyfood-item analyses. So far, the effects of specific dietary phytoestrogens on breast cancer risk and tumor characteristics have been difficult to study because of the lack of a database of phytoestrogen content of foods.

Biologic effects of phytoestrogens

Messina (1999) has reviewed the biologic action and the health effects of phytoestrogens, particularly in cancer, reported from *in vitro*, animal and epidemiologic studies. The prevailing hypothesis is that phytoestrogens exert antiestrogenic effects when placed in an environment high in endogenous estrogens, such as exists in pre-menopausal women, and estrogenic effects when in a low-estrogen environment, such as exists in post-menopausal women. There is some enthusiasm and support for this hypothesis. For example, because of their estrogenic properties, phytoestrogens are being increasingly promoted as the "natural alternative" to estrogen-replacement therapy in postmenopausal women (Gibaldi, 2000). Furthermore, several studies have investigated the hormonal effects of soy isoflavones on endogenous plasma hormones in premenopausal women. They have described antiestrogenic effects of isoflavones, e.g., a decreased urinary excretion of estrogens that reflects the lowering effect of isoflavones on circulating estrogens (Lu, Anderson, Grady, Kohen, & Nagamani, 2000; Nagata, Takatsuka, Kawakami, & Shimizu, 2001; Xu, Duncan, Merz, & Kurzer, 1998).

The estrogenic or antiestrogenic effects of phytoestrogens make them strong candidates to affect hormone-dependent cancers such as breast cancer. In premenopausal women, it is difficult to conclude from laboratory and human studies that

phytoestrogens are necessarily antiestrogenic in breast tissue. If there is any direct anticarcinogenic effect of phytoestrogens on targeted tissues, the literature suggests that phytoestrogens may help to prevent the initiation of cancer cells, rather than inhibiting the growth of existing cancer cells (Ginsburg & Prelevic, 2000; Messina, 1999). To date, there is more evidence supporting the effect of phytoestrogens on breast cancer mediated by hormonal pathways. The action of soy isoflavones to lower endogenous estrogens may reduce exposure of breast cells to estrogens and thus lower breast cancer risk in premenopausal women (Kurzer, 2000). This might explain epidemiologic observations of lowered risk of breast cancer in populations that consume soy.

Molecular mechanisms of phytoestrogen action

Given the complexity of phytoestrogenic effects, it is not surprising that the mechanisms responsible for these effects are only partially resolved. *In vitro* studies provide evidence suggesting that coumestrol and genistein, for example, exert their estrogenic effects through ER-mediated mechanisms (Kuiper et al., 1998; Makela et al., 1994). However, the low serum concentration of phytoestrogens and their weak relative affinity for ERs compared to those of 17β-estradiol do not explain the ER-mediated activity of phytoestrogens (Dopp, Vollmer, Hahnel, Grevesmuhl, & Schiffmann, 1999; Petit, Le Goff, Cravedi, Valotaire, & Pakdel, 1997). The mechanisms by which phytoestrogens exert their antiestrogenic effects are less clear. In addition to competing with endogenous estrogens for binding to ERs and to acting as "natural" selective estrogen-receptor modulators like tamoxifen and raloxifene, phytoestrogens may exert

their antagonistic effects by several other potential mechanisms (Adlercreutz & Mazur, 1997; Makela, Santti, Salo, & McLachlan, 1995). It is noteworthy that phytoestrogens with no considerable affinity for ERs have been reported in recent studies to show nongenomic actions, suggesting that these particular phytoestrogens, as well as others, may exert their biological effects through pathways different from the classical estrogen signaling mechanism (Dopp et al., 1999).

CHAPTER 3

Methods and procedures

Study population

This research is built on the data from two funded prospective studies being conducted at The University of Texas M. D. Anderson Cancer Center, Houston. One is the cohort study "The influence of diet, body size and hormones on breast cancer in premenopausal women" (Dr. Shine Chang, P.I.), conducted in collaboration with Dr. S. Eva Singletary, Department of Surgical Oncology. The other is the case-control study "DNA adduct, p53 mutation, and etiology of breast cancer" (Dr. Melissa Bondy, P.I.).

The participants were premenopausal women recruited between 1998 and 2000. At the time of recruitment, all women were between 18 and 50 years old, had regular menstrual cycles and had been diagnosed with non-metastatic breast cancer (stage IV excluded) within the past six months. The staging of their tumors was based on the American Joint Commission on Cancer system (Harris, Morrow, & Bonadonna, 1993). Among 124 premenopausal cases for whom a dietary questionnaire was available, 19 women diagnosed with stage 0 (*in situ*) breast cancer were excluded from the analysis because ER status of tumors *in situ* was not assessed for clinical purposes. ER status was unknown by pathologists for five patients diagnosed with stage I, II or III breast cancer, so data from these women were also excluded from the analysis. A daily consumption of either less than 500 kilocalories (kcal) or greater than 5,000 kcal was

considered not reliable and one woman reporting a daily intake of 13,165 kcal was excluded. In the data set of 99 patients used for analysis, six women were considered outliers for their high consumption of phytoestrogens, defined by a daily intake greater than the sample mean plus 2 SD. Among the outliers, three were characterized by a high daily intake of genistein resulting from their daily consumption of soy nuts or other soy-based foods; consequently, two of them were also outliers for total isoflavone intake. The three other outliers were for total phytosterols; among them, one woman reported an extreme intake equal to about the sample mean and 20 SD, resulting from her daily consumption of six servings of black tea, which is among the highest sources of β-sitosterol, a phytosterol. Thus, the results presented are for 93 participants after removing the outliers from the data set.

Dietary questionnaire

Dietary assessment was based on the Block-Health Habits and History Questionnaire, a self-administered food frequency questionnaire developed at the National Cancer Institute to assess the consumption of a variety of nutrients, foods, food groups, and dietary supplements. This standardized questionnaire has been adapted in the Department of Epidemiology at M. D. Anderson Cancer Center in order to assess specifically the usual dietary intake of phytoestrogens in the year prior to the diagnosis of cancer (Pillow et al., 1999). From the original 98 food items and 33 nutrient values, the database was enhanced to include 207 foods and 52 nutrient values, including 19

phytoestrogens. These phytoestrogens were selected from the literature according to whether they were commonly consumed in the American population.

Dietary assessment procedure

At the time of study enrollment, all participants were given a self-administered food frequency questionnaire (Appendix A) that they returned by mail. Returned questionnaires were checked for completeness, accuracy and major errors according to a visual check procedure (Appendix B). Patients were queried by mail or by phone call to ensure the provision of correct demographic and dietary information. The Dietary Analysis Personal Computer System (DietSys, version 3.0, National Cancer Institute, Baltimore, MD) was used to enter and double-key questionnaire data, and to identify outliers and keying errors according to an edit check procedure (Appendix C). DietSys was used to generate a nutrient analysis from the data of each individual. Total isoflavone intake, expressed in micrograms (μg), was defined as the sum of genistein, daidzein, formononetin, and biochanin A intake. Total phytosterol intake, expressed in milligrams (mg), was defined as the sum of β-sitosterol, campesterol, and stigmasterol intake. A low genistein intake referred to an intake less than or equal to the median intake of genistein from the sample distribution (63 μg/1000kcal/day), and high genistein intake was defined as greater than the median. Similarly, intake of total isoflavones was divided into high and low consumption based on the median cut point of 188 μg/1000kcal/day, and intake of total phytosterols was divided into high and low consumption based on the median cut point of 89 mg/1000kcal/day.

Statistical design

The independent variables included the mean daily intake of genistein, total isoflavones, and total phytosterols. As specific nutrient intakes can vary with total energy intake because of individual differences in body size, physical activity, and metabolic efficiency, as well as methodological errors that result from assessing diet using a food frequency questionnaire, phytoestrogen intake was adjusted for total calories using the nutrient-density method (Willett & Stampfer, 1998).

Determined by review of pathologic and clinical evaluation reports in the medical records, the dependent variable was ER status, a dichotomous outcome being expressed as either negative or positive, by which the comparisons between the two breast cancer groups (i.e., ER-negative vs. ER-positive) was made. To characterize the sample population, I also considered several demographic variables, including age, ethnicity, education, and household income, as well as body mass index [BMI, weight (kg)/height^2 (m)], the use of oral contraceptives, and the stage of breast cancer at the time of diagnosis.

Descriptive analyses were conducted comparing means, medians, ranges and standard deviations. Differences between the ER-negative and ER-positive groups were tested for statistical significance using non-parametric tests, the Pearson Chi-square test for nominal data, and the Mann-Whitney U-test for ordinal variables and for continuous variables that were not normally distributed (non-parametric analogue to the t-test for 2 independent groups and equivalent to the Wilcoxon rank-sum test). I evaluated the

relationship between ER status and phytoestrogens by comparing the median genistein, total isoflavone, and total phytosterol intakes and by testing the statistical significance of the median differences by the Mann-Whitney U-test. To measure the effect of the independent variables on the outcome variable (i.e., ER status), I created three 2x2 contingency tables where the rows represent cases divided by low and high levels of phytoestrogen intake and the columns represent cases divided by negative and positive ER status. Using these tables, I expressed the strength of the relationship between ER status and the intakes of phytoestrogens as an odds ratio (OR), an estimate used in epidemiology to measure the strength of the association between exposure and outcome. I used the Woolf's method to calculate 95% confidence interval (CI) and $p \leq 0.05$ as the criterion for statistical significance as appropriate. All analyses were performed using programs available in SPSS (version 9.0 for Windows, SPSS, Chicago, IL).

CHAPTER 4

Results

Study population

In this study, there were 63 women (68%) who had ER-positive breast cancer tumors and 30 women (32%) who had ER-negative tumors. Demographics and other descriptive data are presented by ER status in Table 1. The majority of patients were Caucasian (n = 73; 78%), with the remainder being Hispanic (n = 8; 9%), African American (n = 7; 8%), Asian/Pacific Islander (n = 3; 3%), American Indian (n = 1; 1%), and Middle Eastern (n = 1; 1%). The participants were highly educated, with about two thirds having a Bachelor's or higher degree. Consequently, their household income was high, with half of them being above $75,000 per year and only three women (4%) having an annual income under $20,000. According to the American Joint Commission on Cancer staging system, the majority of women (n = 53; 57%) were diagnosed with breast cancer at stage I. The women were between 29 and 50 years of age with a median age of 41 years, and only 26 (28%) were using exogenous estrogens as oral contraceptives at the time of diagnosis. ER-positive and ER-negative groups were similar in demographic characteristics, oral contraceptive use, and stage of breast cancer at the time of diagnosis.

Age and anthropometric data are presented by ER status in Table 2. With a median BMI of 22.9 kg/m^2, most of patients were of normal body size and only two women (2%) were obese (BMI $\geq$ 30 kg/m^2). The prevalence of obesity in this sample was

Table 1

Distribution of Selected Characteristics of Premenopausal Breast Cancer Patients by ER Status

Variable	ER-negative (n = 30)		ER-positive (n = 63)		p-value[a]
	n	%	n	%	
Education[b]					
High school graduate	1	3	8	13	
Vocational/business school	1	3	2	3	
Associate degree/Some college	8	27	15	25	
Bachelor's degree	15	50	21	34	
Master's/doctoral degree	5	17	15	25	0.74
Household income[c]					
Under $20,000	1	4	2	4	
$20,000 to $49,999	9	34	14	25	
$50,000 to $74,999	2	8	13	24	
$75,000 to $100,000	6	23	11	20	
Over $100,000	8	31	15	27	0.99

Note. [a]Two-tailed Mann-Whitney U-test comparing median values from ER-negative and ER-positive case groups. [b]2 missing values in the ER-positive group (n = 61). [c]4 refusals to report income in the ER-negative group (n = 26); 4 missing values and 4 refusals to report income in the ER-positive group (n = 55).

(table continues)

Variable	ER-negative (n = 30)		ER-positive (n = 63)		p-value
	n	%	n	%	
Ethnicity					
White/Caucasian	23	77	50	79	
Other	7[d]	23	13[e]	21	0.77[f]
Use of exogenous estrogens at diagnosis					
Yes	10	33	16	25	
No	20	67	47	75	0.42[f]
Stage of breast cancer at diagnosis					
I	13	43	40	63	
II	15	50	18	29	
III	2	7	5	8	0.11[a]
Body mass index (kg/m^2)					
Normal weight (< 25)	18	60	35	56	
Overweight (25 ≤ BMI < 30)	11	37	27	43	
Obese (≥ 30)	1	3	1	1	0.75[a]

Note. [a]Two-tailed Mann-Whitney U-test comparing median values from ER-negative and ER-positive case groups. [d]4 African Americans, 2 Hispanics, 1 Middle Eastern. [e]3 African Americans, 6 Hispanics, 3 Asians/Pacific Islanders, 1 American Indian/Alaskan. [f]Two-sided Pearson Chi-Square.

Table 2

Range, Median, Mean, and Standard Deviation of Age and Anthropometric Characteristics of Premenopausal Breast Cancer Patients by ER Status

Variable	ER-negative (n = 30)	ER-positive (n = 63)	p-value[a]
Age (years)			
Minimum - Maximum	29 - 50	28 - 50	
Mdn	40.0	41.0	0.34
M	39.6	40.6	
SD	5.6	5.2	
Weight (kg)			
Minimum - Maximum	49.1 - 104.5	43.6 - 129.5	
Mdn	64.8	63.6	0.46
M	65.8	68.8	
SD	13.6	16.5	
Height (m)			
Minimum - Maximum	1.45 - 2.01	1.52 - 1.80	
Mdn	1.62	1.65	0.20
M	1.63	1.65	
SD	0.11	0.07	
Body mass index (kg/m^2)			
Minimum - Maximum	18.6 - 43.4	17.0 - 42.2	
Mdn	22.7	23.8	0.49
M	24.9	25.2	
SD	5.6	5.1	

Note. [a]Two-tailed Mann-Whitney U-test comparing median values from ER-negative and ER- positive case groups.

considerably lower than the national obesity prevalence rate among women [24% (National Institutes of Health National Heart, Lung, and Blood Institute, 1998)] and is likely to reflect the high-education and high-income level of women in the study population, as these demographic characteristics are usually inversely associated with obesity (National Institutes of Health National Heart, Lung, and Blood Institute, 1998). ER-positive and ER-negative groups were similar in age, weight, height and BMI.

Description of dietary intake

Dietary intakes of total energy, genistein, total isoflavones, and total phytosterols are summarized by ER status in Table 3. Total energy intake was similar in the ER-negative and ER-positive groups ($p = 0.54$). Women with ER-positive tumors tended to have a higher genistein intake than their ER-negative counterparts but the difference was not statistically significant ($p = 0.10$). According to the food database, genistein intake was primarily derived from the consumption of soy nuts, tofu, and soy products contained in imitation bacon bits, soy meat substitutes, miso, and breakfast shakes. Three women reported a null dietary intake of genistein. Dietary intake of total isoflavones was comparable between ER-negative and ER-positive women ($p =0.49$). Dietary intake of total phytosterols was lower in women with ER-positive tumors than those with ER-negative tumors, but the difference was still not significant ($p =0.24$). In the sample population, the median value for phytosterol intake was 89 mg/day, which reflects the usual intake in the Western diet (80 mg/day). According to the food database, phytosterol intake was primarily derived from the consumption of green and black tea,

Table 3

Range, Median, Mean, and Standard Deviation of Dietary Factors of Premenopausal Breast Cancer Patients by ER Status

Variable	ER-negative (n = 30)	ER-positive (n = 63)	p-value[a]
Total energy intake (kcal/day)			
Minimum - Maximum	876 - 3744	734 - 4736	
Mdn	1983	1849	0.54
M	2029	1959	
SD	771	798	
Genistein intake (μg/1000kcal/day)			
Minimum - Maximum	0 - 3295	0 - 5970	
Mdn	46	76	0.10
M	187	297	
SD	595	827	
Total isoflavone intake (μg/1000kcal/day)			
Minimum - Maximum	1 - 5326	8 - 10488	
Mdn	191	189	0.49
M	403	562	
SD	959	1453	
Total phytosterol intake (mg/1000kcal/day)			
Minimum - Maximum	25 - 611	24 - 660	
Mdn	112	74	0.24
M	161	137	
SD	143	132	

Note. [a]Two-tailed Mann-Whitney U-test comparing median values from ER-negative and ER- positive case groups.

salad oil, mayonnaise, margarine, dark bread, and green salad. The 2x2 contingency tables presenting the corresponding distributions of low and high intake of phytoestrogens by ER status are given in Table 4.

Odds Ratios

I performed a preliminary analysis among the overall sample of 99 patients, including six outliers for phytoestrogen intake. Women who had a lower dietary intake of genistein (i.e., less than or equal to the sample median intake) compared to those with a greater intake of genistein were 2.3 times more likely to be diagnosed with ER-negative breast cancer compared to ER-positive breast cancer ($OR_{genistein}$ = 2.30, 95% CI = 0.96-5.54). When I focused on total isoflavones, women with a lower intake were 1.29 times more likely to be diagnosed with ER-negative than ER-positive breast cancer ($OR_{isoflavones}$ = 1.29, 95% CI = 0.55-3.02). Although not statistically significant (95% CIs include 1.0), these ORs suggest that a low consumption of genistein or isoflavones is associated with a higher risk of being diagnosed with ER-negative than ER-positive breast cancer. In other words, these ORs suggest a protective effect of a high consumption of genistein or isoflavones against ER-negative breast cancer than against ER-positive breast cancer. Compared to the women who had a greater intake of phytosterols, those with a lower intake were 47% less likely to be diagnosed with ER-negative breast cancer ($OR_{phytosterols}$ = 0.53, 95% CI = 0.22-1.26), which suggests a protective effect of low phytosterol intake against ER-negative breast cancer.

Table 4

Univariate Odds Ratio and 95% Confidence Interval of Negative ER Status Associated With Dietary Intake of Phytoestrogens Among Premenopausal Breast Cancer Patients

Variable	ER-negative (n = 30)	ER-positive (n = 63)	OR[a]	95% CI
Genistein intake[b] (μg/1000kcal/day)				
Low	18	29	1.76	0.73 - 4.25
High	12	34		
Total isoflavone intake[c] (μg/1000kcal/day)				
Low	15	31	1.03	0.43 - 2.46
High	15	32		
Total phytosterol intake[d] (mg/1000kcal/day)				
Low	12	34	0.57	0.24 - 1.37
High	18	29		

Note. 6 outliers for dietary intake are excluded (n = 93). OR = Odds Ratio. CI = Confidence Interval.

[a]ER-positive group is used as the reference group. High dietary intake is used as the reference. [b]"Low" genistein intake refers to intake less than or equal to median intake of genistein from the sample distribution (63 μg/1000kcal/day). [c]"Low" total isoflavone intake refers to intake less than or equal to median intake of total isoflavone from the sample distribution (188 μg/1000kcal/day). [d]"Low" total phytosterol intake refers to intake less than or equal to median intake of total phytosterol from the sample distribution (89 mg/1000kcal/day).

After removing from the analysis six outliers, which were above 2 SD from the sample mean intakes of genistein and phytosterols as described in Study population (Chapter 3), all ORs were attenuated (i.e., closer to 1), and the 95% CI became narrower, yet not statistically significant, as shown in Table 4. The effect of removing the outliers was greater on the OR of genistein. Women with lower intake of genistein and higher intake of phytosterols were more likely to be diagnosed with ER-negative tumors. While there was a trend of association between the risk of having ER-negative breast cancer and intake of genistein, the most potent isoflavone, there was no suggestive association for total isoflavone intake.

Statistical power

To calculate the statistical power of the study to detect an association between ER status and the dietary intake of genistein, I used an on-line statistical power calculator developed by the University of California Los Angeles, Department of Statistics (2001). A normal approximation to the Binomial distribution applies to the case-comparison study design, similar to a case-control design, with nominal variables. The parameters for the 2-sided test were the frequency of the risk factor (i.e., low genistein intake) equal to $(18 + 29) / 93 = 0.5$; the relative risk estimated by the odds ratio equal to 1.76; the significance level $\alpha = 0.05$; the number of cases (ER-negative subjects) equal to 30; and the number of controls (ER-positive subjects) equal to 63. The power (i.e., the probability to reject the null hypothesis) obtained was 0.24 for genistein intake.

To estimate the required sample size for reaching a power of 80% to detect a statistically significant OR of 1.76 of ER-negative status with genistein intake, I used the sample size tables for a two-tailed test on proportions given by Fleiss (1981). The significance level was set equal to $\alpha = 0.05$; the error of type II was $\beta = 4\alpha = 0.20$, and the power was $\underline{P} = 1 - \beta = 0.80$; the proportion of ER-positive patients having low genistein intake was $\underline{p_1} = 29 / 63 = 0.46$ and was rounded to 0.45 for using the tables; the proportion of ER-negative patients having low genistein intake was $\underline{p_2} = 18 / 30 = 0.60$. According to the tables, the sample size necessary would be equal to 186 per ER-status group, or a total of 372 patients compared to the present sample size of 93.

In the present study, the distribution of subjects was not equal in the two ER-status groups. According to Browner, Newman, Cummings, and Hulley (2001), it is useful to estimate a smaller sample size for the ER-negative group when the predictor factor (i.e., high and low genistein intake) and the outcome (i.e., ER positive and negative status) are dichotomous. The recruitment of subjects would be easier and less expensive for a similar statistical power. Given $\underline{n} = 186$ the previous number of ER-negative patients that would be necessary and $\underline{c} = 2$ ER-positive subjects per ER-negative subject, the approximate number of ER-negative subjects was $\underline{n'} = [(\underline{c} + 1) / 2\underline{c}] \times \underline{n} = [(2 + 1) / (2 \times 2)] \times 186 = 140$. In conclusion, the required sample size for a power of 80% would be 326 (186 ER-positive and 140 ER-negative patients) to detect an association equal to 1.76 between ER status and dietary genistein intake.

Using similar calculations, the statistical power to detect an association between ER status and the dietary intake of total isoflavones was almost null (power of 5%). The sample size required to reach a power of 80% would be 2809 (1605 ER-positive and 1204 ER-negative patients). The statistical power to detect an association between ER status and the dietary intake of total phytosterols was the same as for genistein (power of 24%). The sample size required to reach a power of 80% would be the same as for genistein intake (186 ER-positive and 140 ER-negative patients).

CHAPTER 5

Discussion

Summary of findings

The main findings from this study are the suggestive inverse association between risk of ER-negative breast cancer and dietary intake of genistein, and a suggestive positive association for total phytosterol intake. The finding for total isoflavone intake reflected that for genistein intake, but was not as large in magnitude. In other words, women who had a greater intake of genistein and a lower intake of total phytosterols in the year before their diagnosis of breast cancer tended to be at lower risk of being diagnosed with an ER-negative tumor relative to being diagnosed with an ER-positive tumor. While the magnitude of the estimates for these two associations showed an almost two-fold difference in risk, 95% CIs included 1.0 and thus, the null hypothesis (i.e., that women with ER-negative breast cancer and those with ER-positive tumor had the same prediagnostic dietary intake of genistein, isoflavones and phytosterols) fails to be rejected at the $\alpha = 0.05$ level.

Genistein and isoflavones in cancer

Isoflavones, including the most potent phytoestrogen genistein, are hormone-like diphenolic phytoestrogens found in high concentration in soybeans and soy-based foods. In addition to having weak estrogenic and antiestrogenic effects on ER-mediated estrogenic pathways, isoflavones are thought to exert other properties via mechanisms

not involving the ERs. These properties were found to be important in cancer, including inhibition of cell proliferation, inhibition of angiogenesis, and inhibition of enzymes such as tyrosine-specific protein kinases, topoisomerase II and other enzymes involved in signal transduction (Messina et al., 1994).

The studies of genistein and isoflavone intake in relation to cancer have mainly addressed the reduced risk of cancer of the breast, prostate, and colon associated with a higher consumption of soy-based foods. To date, no studies have investigated the effects on breast cancer risk of specific isoflavones. However, some epidemiological studies have investigated isoflavones in relation to prostate cancer, which, like breast cancer, is a hormone-related cancer. Although the data are limited and inconsistent in their beneficial or deleterious effects with regard to prostate cancer, a large cohort study conducted in Hawaii reported a protective dose-response trend for tofu consumption against risk of prostate cancer (Severson, Nomura, Grove, & Stemmermann, 1989). The only study to look at the effects of specific phytoestrogens on prostate cancer risk by using the same dietary phytoestrogen database as in the present study reported a slight protective effect of genistein against prostate cancer (Strom et al., 1999). Overall, the literature suggests a protective effect of isoflavones against the development of some hormone-dependent cancers, possibly mediated through their anti-estrogenic effects on steroid levels that may affect cancer promotion.

Previous dietary studies related to ER status in breast cancer have indicated a protective effect of fiber, vitamin A, carotenoids, and vitamin B6 against ER-negative

breast cancer (Ingram et al., 1992; Rock et al., 1996). In the present study, a suggestive protective effect was found against ER-negative status for genistein. However, total isoflavone intake, computed from genistein, daidzein, formononetin and biochanin A intake, was weakly related to ER status in this study. As genistein is the most potent isoflavone in terms of estrogenic properties, the predominant effect of genistein may have been attenuated in the analysis of total isoflavones of which genistein was one of four isoflavones considered. This could explain the limited association with ER status for total isoflavones in this study, similar as in controlled trials showing no significant hormonal effect of dietary isoflavones (Ginsburg & Prelevic, 2000).

Mechanisms for genistein and isoflavones effects on ER status

As the biochemical structure of genistein and isoflavones resembles to some extent those of endogenous estrogens, it is not surprising that the ER-mediated estrogenic activity of isoflavones has been reported, albeit weaker than endogenous estrogens. Since ER expression is estrogen dependent, three laboratory studies have examined the molecular effects of isoflavones on ER expression in the ER-positive human breast cancer cell line MCF-7 (Sathyamoorthy & Wang, 1997; Wang, Sathyamoorthy, & Phang, 1996; Zava & Duwe, 1997). Long-term exposure of MCF-7 cells to either genistein, daidzein, or equol (its derived compound) resulted in a decreased ER expression similar to the down-regulation by 17β-estradiol. These results suggest that, in absence of endogenous estrogens, isoflavones have the same estrogenic potency for regulating ER expression as estrogens. Consequently, it is reasonable to hypothesize that

in post-menopausal women who have low levels of endogenous estrogens, isoflavones could increase ER expression in ER-negative breast cancer cells by their estrogenic effect. Thus, consumption of isoflavones might increase the likelihood of a breast cancer tumor to be ER-positive in a post-menopausal woman. It would be consistent with the hypothesis that the estrogenic properties of isoflavones can serve as a food-based alternative to estrogen-replacement therapy, even at the low concentrations provided by dietary intake.

Owing to the high levels of endogenous estrogens in premenopausal women and the relative low estrogenic potency of isoflavones, the hypothesis of ER regulation by the estrogenic action of isoflavones may not be relevant to premenopausal breast cancer. First, isoflavones have been reported to exert an antiestrogenic activity in presence of estrogens either by competing with estrogens for ER binding and reducing their estrogenic activity, or by decreasing concentrations of circulating free estrogens (Adlercreutz & Mazur, 1997; Lu et al., 2000; Makela et al., 1995; Nagata et al., 2001; Xu et al., 1998). In either case, the antiestrogenic effects of isoflavones might regulate ER expression in premenopausal women and result in an increased expression of ERs due to a decreased exposure to estrogens, by using the same molecular pathways involved in the down-regulation of ERs by 17β-estradiol. Secondly, cellular studies have suggested other mechanisms for the antiestrogenic effects of isoflavones independently from the estrogen signaling pathways (Dopp et al., 1999). These studies are particularly relevant to explain the possible effect of isoflavones in stimulating ER

expression in ER-negative breast tissue. It is likely that, in premenopausal women, isoflavones exert their regulative action on ER expression via non-ER mediating mechanisms.

Phytosterols in cancer

Phytosterols, plant sterols, are another group of phytoestrogens, structurally similar to cholesterol, except that they contain substitutions at the C-24 position of the sterol side chain (Ling & Jones, 1995). The most common dietary phytosterols are β-sitosterol, campesterol, and stigmasterol. Phytosterols are the most abundant phytoestrogens with amounts in the milligram range, being found in fruits and vegetables. The dietary sources with the highest concentrations of phytosterols are unrefined plant oils, seeds, and nuts. Phytosterols are not new to the human diet, although the usual intake in the Western diet (80 mg/day) is certainly lower than that of our ancestors and is lower than the vegetarian (345 mg/day) and Japanese (400 mg/day). Phytosterols can be consumed in large quantities without any apparent toxicity concerns, and the only contraindication for consumption is among individuals with an inherited condition interfering with phytosterol metabolism (i.e., phytosterolemia) (Jones & Raeini-Sarjaz, 2001).

Because the structure of phytosterols is similar to that of cholesterol, most of the research on the effects of phytosterols has addressed cholesterol metabolism and atherosclerosis (Moghadasian & Frohlich, 1999). In addition to protection from cardiovascular diseases, phytosterols have been suggested to possess several other

therapeutic activities, including potent anti-inflammatory, antibacterial, and antifungal properties (Ling & Jones, 1995). Although the estrogenicity of phytosterols has been hypothesized, *in vitro* and animal assays have not demonstrated any estrogenic effect of phytosterols (Awad & Fink, 2000).

Other experimental and epidemiological studies have suggested that phytosterols possess protective properties from several types of cancer. Experimentally, phytosterols, particularly β-sitosterol, were shown to have anti-carcinogenic effects by inhibiting cellular growth, retarding spread of disease and stimulating apoptosis in colon, prostate, and breast cancer cell lines as well as in animal models of cancer (Awad, Downie, Fink, & Kim, 2000; Awad & Fink, 2000; Ling & Jones, 1995). Four recent case-control studies presenting a nutrient analysis of phytosterols in relation to cancer have shown a significant protective effect of phytosterols against cancer of the stomach (De Stefani et al., 2000), endometrium (McCann et al., 2000), lung (Mendilaharsu, De Stefani, Deneo-Pellegrini, Carzoglio, & Ronco, 1998), and breast (Ronco et al., 1999). There is supporting evidence for the protective effect of consumption of tea, particularly green tea, the richest dietary source in phytosterols, against cancer of the breast, stomach, esophagus, and lung (Nakachi et al., 1998; Torres-Sanchez, Lopez-Carrillo, Lopez-Cervantes, Rueda-Neria, & Wolff, 2000). However, two older studies argued against the etiological relationship between phytosterols and breast cancer, by reporting no difference of phytosterol concentration between normal and malignant breast tissues (Haddad, Couranz, & Avioli, 1970; Mellies, Ishikawa, Glueck, & Crissman, 1977). In a

recent case-control study on prostate cancer, Strom et al. (1999) found an increased risk of prostate cancer with campesterol and stigmasterol. Overall, phytosterols are likely to protect against cancer, but it is suggested that their antiproliferative and chemoprotective effects may be tissue specific.

The present findings are the first reported between phytosterol intake and ER status. Unlike the suggested protective effects of phytosterols in risk of developing breast cancer, these data suggest an increased risk of ER-negative tumors to be associated with total phytosterol consumption. Effects of phytosterols have been selectively reported in cancer, β-sitosterol being especially potent in preventing colon, prostate and breast cancer, while campesterol and stigmasterol intake being associated with a increased risk of prostate cancer (Awad, Chan, Downie, & Fink, 2000; Awad & Fink, 2000; Strom et al., 1999). Thus, studies that evaluate the separate effects of individual plant sterols are necessary to clarify phytosterol properties in regard to breast cancer.

Mechanisms for phytosterols effects on ER status

According to the literature, phytosterols do not bind to ERs and have no estrogenic effects either *in vivo* or *in vitro*. They do not stimulate expression of human ERs, at least in yeast (Awad & Fink, 2000). Therefore, the effect of phytosterols on ER expression, and consequently on ER status, is independent of the classic down-regulation of ERs by estrogens. Other indirect pathways must be considered.

The specific anti-carcinogenic mechanisms of phytosterols are not well known but several theories have been proposed. In prostate cancer, one hypothesis suggests that

phytosterols inhibit membrane-bound enzymes involved in the metabolism of androgens and estrogens (Awad & Fink, 2000). A similar hypothesis proposes that phytosterols may inhibit membrane-bound enzymes (e.g., aromatase) involved in estrogen metabolism in the ovaries of premenopausal women, thus decrease the estrogen production and the exposure to circulating levels of estrogens of breast tissues, and protect against breast cancer. However, in presence of a down-regulation of ERs by estrogens, this hypothesis of decreased estrogen exposure associated with phytosterol consumption would not explain a decreased ER expression and consequently a higher risk of ER-negative status associated with phytosterol consumption in the present study.

Because phytosterols have a similar structure to cholesterol that is a component of biological membranes, incorporation of phytosterols into membranes has been studied. Phytosterols have the potency to decrease the concentration of sphingomyelin, a phospholipidic constituent of membranes, by 50% (without affecting total phospholipid concentration) and to modify the fluidity of cell membranes without altering their integrity (Awad & Fink, 2000). Through these functional alterations of cell membranes, phytosterols might affect a large number of cellular regulations, including estrogen metabolism, ER function or ER expression.

It is well established that phytosterols lower plasma total and low-density lipoprotein cholesterol levels in a dose-response manner, mainly by inhibiting intestinal cholesterol absorption through their competitive binding to cholesterol receptors (Moghadasian & Frohlich, 1999). Alterations in cholesterol absorption and metabolism affect

composition of cell membranes in cholesterol. It is possible that the effect of phytosterols on the metabolism of cholesterol itself, in addition to the sphingomyelin cycle alteration, would also indirectly affect ER expression regulation.

The mechanisms by which phytosterols influence breast cancer characteristics like ER status are unclear and require further investigation. This is especially urgent because the public has been advised of the effectiveness of phytosterols in reducing blood cholesterol and colon cancer risk, and to consume phytosterol-enriched oil products.

Dietary supplementation in phytoestrogens

It is clear from *in vitro* and animal studies that the beneficial effects of some phytoestrogens are likely to be achieved only at doses much higher than those that can be obtained from eating plants. In October 1999, the United States Food and Drug Administration approved the health claim that soy protein was useful in reducing the risk of coronary heart disease. Phytosterols received the same approval in September 2000 and are currently marketed as functional foods in spreads and salad dressings. One can view the addition of phytosterols to foods as a simple replacement of what once existed in our ancestral diets (Jones & Raeini-Sarjaz, 2001). However, the approach of supplementing foods with phytoestrogens or genetically manipulating plant strains to enrich them in beneficial phytochemicals, as suggested by Rowland (1999), has to be considered carefully until we know more about the safety of these interventions. People with or at high risk of chronic diseases like cancer are a population particularly at risk of possible side effects. At least, dietary changes in the Western countries should be

recommended to maximize intake of the most bioactive and beneficial components. Encouraging American people to eat more vegetables would probably not raise their phytoestrogen consumption up to the level attained in some Asian countries, but it would at least increase their phytoestrogen intake and be beneficial in regard to cancer and other chronic diseases.

Study limitations

Like any epidemiologic research, this study has several methodological limitations, particularly inherent to all studies using food-frequency questionnaires. As participants were asked to report their diet over the year before their diagnosis of breast cancer, only newly-diagnosed breast cancer patients were included into the study to minimize recall bias. Since a food frequency questionnaire is directed to the usual food intake over an extended period of time, the dietary data have some inherent estimation error in measuring true food intake. In addition to the validity of reporting of food intake, the estimated intake of a specific nutrient depends on the number of foods contributing to the intake of that nutrient in the nutrition questionnaire. To the author's knowledge, the comprehensive food database used in this dietary analysis is the only instrument that quantifies dietary intake of specific isoflavones and phytosterols and that includes an open-ended section to specifically report soy-based foods, as well as a variety of Mexican and Asian foods rich in phytoestrogens and consumed by a large population in the Southwest region. Additional data adjusting for the absorption of phytoestrogens and their bacterial conversion to active metabolites, and for potential hormone-

modulating effects of other nutrients not captured by the nutrient database, would enhance the ability of the nutrition questionnaire to quantify phytoestrogen exposure.

The greatest limitation of the present study was the small sample size. As a result, this study has limited statistical power to detect an association between phytoestrogen exposure and ER status (see in Chapter 4, Statistical power). A study 3.5 times larger (sample size of 326 participants) would be able to detect a similar association between ER status and dietary intake of genistein and phytosterols with a power of 80%. Although the sample size was small, the present study is one of the first to address this issue.

Confounding is always an inherent source of error in case-comparison studies. Instead of an univariate analysis, a multivariate regression model including known risk factors for ER status would minimize the effects of potential confounders, but it would require a larger sample size. Although the two ER-status groups were not matched, the effect of potential confounding factors was likely to be limited, as the groups were similar in their demographic and other characteristics collected in this study.

Finally, the ability of the study to detect an association between phytoestrogen exposure and ER status was limited by the dietary pattern of the study population. The Western diet (i.e., diet high in fat and proteins and low in fiber and complex carbohydrates) is known to be low in soy-based foods and in vegetables compared to the Asian diet. As the high/low levels of phytoestrogen intake and outliers for phytoestrogens were defined relatively to the sample intake, the cut points would be

different in another population. Particularly, the phytoestrogen median intake would be expected to be higher in Asian populations. The phytoestrogen intake of some individuals that was classified as high in this study is likely to be considered as low if a similar study was conducted in Japan, Singapore or China. Similarly, the six outliers for high genistein and phytosterol consumption would not be excluded and the analysis would take into account their consumption that indeed reflects real dietary patterns. However, while the homogeneity in the dietary intake of the sample population was likely to mask any difference in phytoestrogen consumption, the effects detected in this study can be considered as particularly important. Larger studies including populations with a large interval of phytoestrogen intake are needed to refine the role of phytoestrogens in health in general.

CHAPTER 6

Conclusion

In this analysis of data obtained from 93 premenopausal breast cancer patients, a low consumption of genistein and a high consumption of total phytosterols put women at greater risk of developing ER-negative tumors than ER-positive tumors, although no association was statistically significant. These results are suggestive of a protective effect of a high dietary intake of genistein and a low dietary intake of phytosterols against ER-negative breast cancer. Although these findings were not statistically significant, they are consistent with the possibility that consumption of foods rich in specific phytoestrogens may modulate breast cancer characteristics.

While the present findings are suggestive, the analysis was limited by the small number of women in the study, but a larger sample size might yield statistically significant effects of phytoestrogens on ER status. Confirmation by studies with larger populations, as well as studies with post-menopausal women, is needed. One strength of the analysis was the use of a food database that contained a comprehensive list of phytoestrogens. Thus, future studies can include evaluation of other phytoestrogens in relation to ER status in breast cancer patients.

Vegetable foods contain numerous types of phytoestrogens for some of which biological activities have been shown. However, our knowledge of the effects of dietary phytoestrogens on human health is still limited and taking high doses of phytoestrogen

supplements should not be recommended until we know more about the metabolism of phytoestrogens. Further work is needed to clarify the mechanism for the estrogenic or antiestrogenic activity of phytoestrogens, particularly in estrogen-sensitive tumors. If ER status were to be demonstrated to be modifiable through diet, then this finding would provide important opportunities for developing new cancer prevention dietary recommendations. Although the scientific evidence is incomplete and controversial regarding the benefit of certain plant components in cancer prevention, it is not premature to recommend a diet rich in fruits and vegetables, in part because such diets have not been shown to be harmful, but also because fruits and vegetables contain a number of micronutrients that are known to be protective against cancer and other diseases.

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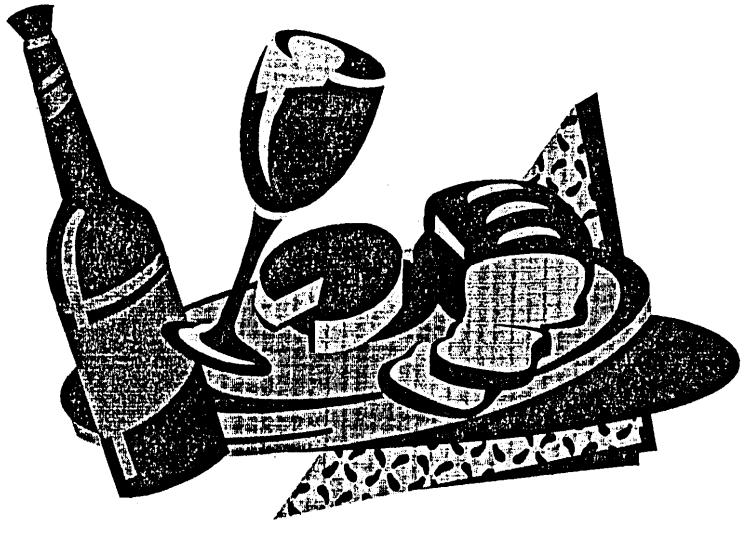
APPENDIX A

Food frequency questionnaire

FOOD QUESTIONNAIRE



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FOOD QUESTIONNAIRE

This questionnaire asks about your usual food intake during the last year. Please answer each question as best you can - estimate if you are not sure of an answer. Some questions can be answered by a check mark while others require a numerical answer. There are no right or wrong foods on this questionnaire. Foods are listed on the questionnaire in order to study the relationship (if any) between diet, food habits, and health. Brand names are used only to help identify different types of food products.

Today's date: ___ ___/___ ___/___ ___ ___ ___ *(month/day/year)*

1. Your name (please print):

_____
(First) *(Last)*

2. Gender: _____ Male _____ Female

If female, are you pregnant or breast-feeding? _____ No _____ Yes

3. Age (years): ___ ___

4. Height: ___ **feet** ___ ___ **inches**

5. Weight:

___ ___ ___ **Current weight (pounds)**
___ ___ ___ **Usual weight last year (pounds)**
___ ___ ___ **Usual weight five years ago**

6. Were you on a special diet in the last year or prior to your current illness? _____ **No** *(If no, skip to next page)* _____ **Yes**

If yes, what type of diet?

_____ Weight loss
_____ Medical condition
_____ Vegetarian
_____ Low salt
_____ Low fat or low cholesterol
_____ Weight gain
_____ Other _____
(describe)

PAGE: 1 IDNUMBER: _____

NOTE:

Cases: All of the following questions are about foods or supplements you normally ate in the year **prior to your diagnosis.**

Controls: All of the following questions are about the foods or supplements you normally ate during the **past year.**

7. About how often did you eat the following foods from restaurants or carry-outs? Remember to think about all meals (breakfast, lunch, dinner or snacks).

(Check answers)

Restaurant Food	Almost every day	2-4 times a week	Once a week	1-3 times a month	5-10 times a year	1-4 times a year	Rarely or Never
Fried chicken							
Burgers							
Pizza							
Chinese food, Thai or other Asian food							
Mexican food							
Fried fish							

8. During the last year or prior to your current illness, did you take any vitamin or mineral supplements?

_____ No _____ Yes, fairly regularly _____ Yes, but not regularly

(If no, skip to page 10)

9. If you took multiple vitamins, did you usually take types that:

_____ Contained minerals (iron, zinc, etc.) _____ Did not contain minerals _____ Don't know

10. Did you take the following types of <u>multiple vitamin</u> pills or tablets? If so, how many pills or tablets did you usually take? How often and for how many years did you take them?

- ***Multiple vitamins like regular One-A-Day and Centrum or multiple vitamins that are similar to One-A-Day or Centrum:***

  _____ No _____ Yes ___ ___ per _____
  (number) (Specify: day, week, or month)

  Number of years multiple vitamins like regular One A Day or Centrum were taken:

  ___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

  How often did you take multiple vitamins like regular One A Day or Centrum vitamins <u>with meals</u>?

  _____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Multiple vitamins like Stress Tabs or multi-vitamins that are similar to Stress Tabs:***

  _____ No _____ Yes ___ ___ per _____
  (number) (Specify: day, week, or month)

  Number of years multiple vitamins like Stress Tabs were taken:

  ___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

  How often did you take multiple vitamins like Stress Tabs <u>with meals</u>?

  _____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Therapeutic mulitple vitamins like Theragran or similar types:***

_____ No _____ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

Number of years therapeutic multiple vitamins like Theragan were taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take therapeutic multiple vitamins like Theragan <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

11. Did you take any single vitamin or mineral supplements in addition to or in place of the multiple vitamin supplements listed on previous pages? (*If yes, describe them below but <u>do not include supplements which were part of your multiple vitamin(s).)</u>*

_____ No *(If no, skip to question 12 on page 8)* _____ Yes

- ***Vitamin A (not beta-carotene):***

___ No ___ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many I.U.'s per pill?

___ 100 IU ___ 200 IU ___ 400 IU ___ 1000 IU

___ 5000 IU ___ 10,000 IU ___ 25,000 IU ___ 50, 000 IU

How often did you take vitamin A (not beta-carotene) <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Beta-carotene:***

___ No ___ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

How many years beta-carotene taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many micrograms per pill?

___ 6,000 mcg ___ 10,000 mcg ___ 15,000 mcg ___ 20,000 mcg

___ 25,000 mcg ___ 30,000 mcg ___ 50,000 mcg ___ 85,000 mcg

How often did you take beta-carotene <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Vitamin C:***

___ No ___ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

How many years vitamin C taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many milligrams per vitamin C pill?

___ 100 mg ___ 250 mg ___ 500 mg ___ 1000 mg

___ 1500 mg ___ 2000 mg ___ 3000 mg ___ 5000 mg

How often did you take vitamin C <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Vitamin E:***

___ No ___ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

Number of years vitamin E taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many IU's per vitamin E pill?

___ 100 IU ___ 200 IU ___ 400 IU ___ 600 IU

___ 800 IU ___ 1000 IU ___ 20,000 IU ___ 40,000 IU

How often did you take vitamin E <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Calcium, Dolomite or Tums®:***

____ No _____ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

How many years calcium, dolomite or Tums® taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many milligrams per calcium, dolomite or Tums® pill? _____

___ 100 mg ___ 250 mg ___ 300 mg ___ 500 mg

___ 600 mg ___ 800 mg ___ 1000 mg ___ 1200 mg

How often did you take calcium, dolomite or Tums® <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

Does your calcium supplement contain Iron? ___ Yes ___ No___ Don't know

Does your calcium supplement contain Vitamin D? ___ Yes ___ No___ Don't know

Does your orange juice contain calcium? ___ Yes ___ No___ Don't know

- ***Zinc:***

_____ No _____ Yes ___ ___ **per** _____
(number) (Specify: day, week, or month)

How many years zinc taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many milligrams per zinc pill?

___ 10 mg ___ 15 mg ___ 20 mg ___ 25 mg

___ 30 mg ___ 50 mg ___ 100 mg ___ 120 mg

How often did you take zinc <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Iron:***

_____ No _____ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

How many years iron taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many milligrams per iron pill?

___ 30 mg or less ___ 31-50 mg ___ 51-100 mg ___ More than 100 mg

How often did you take iron <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Selenium:***

_____ No _____ Yes ___ ___ per _____
(number) (Specify: day, week, or month)

Number of years selenium taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How many micrograms per selenium pill? ___ ___ ___

How often did you take selenium <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

12. Did you take any other supplements such as folic acid, B vitamins with C, antioxidant supplements, cod liver oil, yeast, or other supplement products?

_____ No (If no, skip to page 10) _____ Yes

- ***Supplement (name):*** _____

___ ___ per _____
(number) *(Specify: day, week, or month)*

Strength (amount) of each pill or tablet? _ _ _ _ _ **Units (mcg, mg, IU, or grams?)**_____

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take this supplement <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Supplement (name):*** _____

___ ___ per _____
(number) *(Specify: day, week, or month)*

Strength (amount) of each pill or tablet? _ _ _ _ _ **Units (mcg, mg, IU, or grams?)**_____

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take this supplement <u>with meals</u>?

_____ Never _____ Sometimes _____ Usually _____ Almost always

- ***Supplement (name):*** ______________________

___ ___ per ______________________
(number) *(Specify: day, week, or month)*

Strength (amount) of each pill or tablet? __ __ __ __ __ **Units (mcg, mg, IU, or grams?)**_________

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take this supplement with meals?

______ Never ______ Sometimes ______ Usually ______ Almost always

- ***Supplement (name):*** ______________________

___ ___ per ______________________
(number) *(Specify: day, week, or month)*

Strength (amount) of each pill or tablet? __ __ __ __ __ **Units (mcg, mg, IU, or grams?)**_________

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take this supplement with meals?

______ Never ______ Sometimes ______ Usually ______ Almost always

- ***Supplement (name):*** ______________________

___ ___ per ______________________
(number) *(Specify: day, week, or month)*

Strength (amount) of each pill or tablet? __ __ __ __ __ **Units (mcg, mg, IU, or grams?)**_________

Number of years taken:

___ Less than 1 year ___ 1-2 years ___ 3-5 years ___6-9 years ___ 10 years

How often did you take this supplement with meals?

______ Never ______ Sometimes ______ Usually ______ Almost always

13. Who usually purchased foods that you ate?
(Check only one)

_____ Self _____ Spouse/Significant Other _____ Other

14. How often did you eat the skin on chicken? *(Check answer)*

_____ Seldom/Never _____ Sometimes _____ Often/Always

15. How often did you eat the fat on meat? *(Check answer)*

_____ Seldom/Never _____ Sometimes _____ Often/Always

16. How many times a day, week, month, or year did you usually use fat or oil in cooking? For example, in frying eggs, meat, or vegetables?

___ ___ per _____ _____ Rarely or never cook with fat or oil
(number) *(specify: day, week, or month)*

17. In general, how cooked was the meat you usually ate in the last year?

_____ Rare _____ Medium _____ Well done

18. What kinds of fat or oil did you usually use in cooking (to fry or stir-fry)?
(Check the one or two types used most often.)

_____ Don't know or don't cook
_____ Corn oil or vegetable oil
_____ Lard, fatback, bacon fat
_____ Crisco/solid shortening
_____ Stick margarine
_____ Soft tub margarine
_____ Low calorie margarine
_____ Butter
_____ Olive oil or canola oil
_____ Non-stick spray or no oil

19. What kinds of fat did you usually add to vegetables, potatoes, etc. at the table?
(Check the one or two types used most often.)

_____ Don't add fat

_____ Lard, fatback, bacon fat

_____ Crisco/solid shortening

_____ Stick margarine

_____ Soft tub margarine

_____ Low calorie margarine

_____ Butter

_____ Whipped butter

_____ Olive oil

_____ Non-stick spray or no oil

20. Not counting salad or potatoes, about how many servings of vegetables did you usually eat per day, week, or month?

___ ___ Servings per _____
(specify: day, week, or month)

_____ Rarely or never ate vegetables not counting salad or potatoes

21. Not counting juices, about how many servings of fruits did you usually eat per day, week, or month?

___ ___ Servings per _____
(specify: day, week, or month)

_____ Rarely or never ate fruits

22. About how many servings of <u>cold</u> cereal did you usually eat per day, week, or month?

___ ___ Servings per _____
(specify: day, week, or month)

_____ Rarely or never ate cold cereals

23. Which cold cereal did you usually eat <u>most</u> often? *(List only one cereal)*

Brand (if known): _____ Cereal Name: _____

24. About how many glasses of milk (or chocolate milk) did you usually drink per day, week, or month? *(do not include milk on cereal)*

___ ___ Servings per _____
(specify: day, week, or month)

_____ Rarely or never ate milk (or chocolate milk)

25. When you ate the following foods, how often were they the fat free version of the food item?
(Check answers)

- ***Cheese:***

_____ Always Fat Free _____ Sometimes Fat Free _____ Rarely or Never Fat Free

- ***Ice cream / Yogurt:***

_____ Always Fat Free _____ Sometimes Fat Free _____ Rarely or Never Fat Free

- ***Salad dressing /Mayonnaise:***

_____ Always Fat Free _____ Sometimes Fat Free _____ Rarely or Never Fat Free

- ***Cookies / Cake:***

_____ Always Fat Free _____ Sometimes Fat Free _____ Rarely or Never Fat Free

- ***Other food items: (Specify item:_____)***

_____ Always Fat Free _____ Sometimes Fat Free _____ Rarely or Never Fat Free

26. When you ate the following foods, how often were they the low fat (not fat free) version of the food item?
Check answers)

- ***Cheese:***

_____ Always Low Fat _____ Sometimes Low Fat _____ Rarely or Never Low Fat

- ***Ice cream / Yogurt:***

_____ Always Low Fat _____ Sometimes Low Fat _____ Rarely or Never Low Fat

- ***Salad dressing /Mayonnaise:***

_____ Always Low Fat _____ Sometimes Low Fat _____ Rarely or Never Low Fat

- ***Cookies / Cake:***

_____ Always Low Fat _____ Sometimes Low Fat _____ Rarely or Never Low Fat

- ***Other food items: (Specify item:_____)***

_____ Always Low Fat _____ Sometimes Low Fat _____ Rarely or Never Low Fat

The following questions on this page are about certain foods that might be eaten at the same time.

27. When you ate fish, how often did you eat it with lemon juice?

_____ Never eat fish
_____ Never eat fish with lemon
_____ Sometimes
_____ Usually
_____ Almost always

28. When you ate bacon, how often did you eat it with fruit juice and/or tea?

_____ Never eat bacon
_____ Never eat bacon with fruit juice or tea
_____ Sometimes
_____ Usually
_____ Almost always

29. When you ate cured meats, such as ham, salami, etc., how often did you eat it with tomato, fruit juice, tea or beer?

_____ Never eat cured meats
_____ Never eat cured meats with tomato, fruit juice or beer
_____ Sometimes
_____ Usually
_____ Almost always

30. When you drank beer, how often did you drink it with meals?

_____ Never drink beer
_____ Never drink beer with meals
_____ Sometimes
_____ Usually
_____ Almost always

31. When you eat vegetables, are they raw or cooked? ***(Check all answers that apply)***

_____ Seldom or Never eat vegetables **either** raw or cooked

_____ Seldom or Never eat **raw** vegetables
_____ Sometimes eat **raw** vegetables
_____ Usually eat **raw** vegetables rather than **cooked** vegetables
_____ Almost always eat **raw** vegetables rather than **cooked** vegetables

_____ Seldom or Never eat **cooked** vegetables
_____ Sometimes eat **cooked** vegetables
_____ Usually eat **cooked** vegetables rather than **raw** vegetables
_____ Almost always eat **cooked** vegetables rather than **raw** vegetables

Important Information
Instructions and Example Below Shows How to Complete Food Questions On The Following Pages

The next section of the questionnaire asks *about* how often you *usually* ate certain types of foods. If you have never had cancer, the food questions are about the foods you usually ate in the last year (12 months). If you have had cancer, answer about the foods you usually ate in the year prior to your diagnosis. It is not necessary to recall the exact number of times each food was eaten. Your best guess as to whether you ate the food items during that time and about how often and how much is all that is needed.

FIRST: Put a NUMBER in the most appropriate column to indicate HOW OFTEN the food items were eaten. For example, if bananas were eaten twice a week, the number two would be written in the "Week" column. If the food item was rarely or never eaten, place a check mark (✓) in the "Rarely or No" column and do not complete the serving size column for that food item.

SECOND:. Place a check mark (✓) in the serving size columns to show how your usual serving size compared to food servings eaten by other people of your age and gender.

NOTE: Please DO NOT SKIP foods. Be sure your answer is in the correct column.

In the example below, the following foods were usually eaten during the year.

a.) One medium serving of cantaloupe once a week.
b.) One half grapefruit about twice a month.
c.) A small serving of sweet potatoes about 3 times a year.
d.) A large hamburger or cheeseburger about four times a week.
e.) Never ate liver.

Example Shows How to Record Answers:

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column	SMALL	MEDIUM	LARGE
Cantaloupe		*1*				1/2 fruit		✓	
Grapefruit			*2*			1/2 fruit		✓	
Sweet potatoes, yams				*3*		1 cup	✓		
Hamburgers, cheeseburgers		*4*				1 medium patty			✓
Liver					✓	4 oz.			

PAGE: 14 IDNUMBER: _____

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Foods such as fruits and vegetables refer to all forms of the foods such as fresh, canned, or frozen. Note if. seasonal food item.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column to show how your *usual* serving size compares to other people of your age and gender.	SMALL	MEDIUM	LARGE
Apples or applesauce (in any form)						1 whole or 1/2 cup			
Apple juice or grape juice						6 fl oz glass			
Pears (in any form, fresh, canned, frozen)						1 whole or 1/2 cup			
Bananas						1 whole or 1/2 cup			
Peaches (in any form)						1 whole or 1/2 cup			
Apricots (in any form)						1 whole or 1/2 cup			
Cantaloupe (fresh or frozen)						1/4 medium			
Watermelon (in season)						1 medium slice	S	M	L
Other melons such as honeydew and casaba						1/4 medium			
Prunes or prune juice						1/2 cup			
Strawberries (fresh or frozen type)						1/2 cup			
Cherries (in any form)						1/2 cup			
Cranberry juice cocktail, cranberry sauce, cranberries (in any form)						1/2 cup or 6 fl.oz.			
Blueberries (in any form)						1/2 cup			
Other berries such as raspberries, blackberries, boysenberries						1/2 cup			
Sunny Delight, Tang, or Start breakfast juice						8 fl. oz. glass			
Hi-C®, Kool-Aid®, or other fruit drinks with added vitamin C						8 fl. oz. glass			
Orange juice (100% juice)						6 fl. oz. glass			
Oranges						1 medium			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Grapefruit juice (100% juice)						6 fl. oz. glass			
Grapefruit						1/2 of whole fruit			
Grapes						1 cup			
Plums						1 whole or 1/2 cup			
Pineapple or pineapple juice						6 fl. fl. oz. or 1/2 cup			
Diet soft drinks—caffeine free						12 fl. oz. can or bottle			
Diet soft drinks with caffeine						12 fl. oz. can or bottle			
Regular soft drinks (**non-diet**, caffeine free)						12 fl. oz. can or bottle			
Regular soft drinks (**non-diet,** with caffeine)						12 fl. oz. can or bottle			
Beer						12 fl. oz. can or bottle			
Red wine						1 medium glass			
White wine						1 medium glass			
Bourbon						1 shot			
Other liquors						1 shot			
Decaffeinated coffee						1 cup			
Coffee (not de-caffeinated)						1 cup			
Green tea						1 medium			
American Ginseng tea						1 medium cup			
Other herbal teas						1 medium cup			
Black teas such as Lipton®, Nestea®, or English tea						1 medium			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year do you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods during the last year. If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Sweetened bottled waters or bottled iced teas						12 fl oz. bottle			
Non dairy creamer in coffee or tea						1 tablespoon			
Cream (real) or Half-and-Half in coffee or tea						1 tablespoon			
Milk in coffee or tea						1 tablespoon			
Artificial sweetener in coffee or tea (Which pack?: Pink ___ Blue _____)						1 teaspoon equivalent			
Artificial sweetener added to *other* foods (Which pack?: Pink ___ Blue _____)						1 teaspoon equivalent			
Sugar in coffee or tea						2 teaspoons			
Lemon in tea (squeezed or lemon juice added to tea)						1 wedge equivalent			
String beans, green beans						1/2 cup			
Snow pea pods (Chinese pea pods)						10 pods or 1/2 cup			
Green peas						1/2 cup			
Black-eyed peas						3/4 cup			
Squash						1/2 cup			
Tomatoes						1 whole or 1/2 cup			
Salsa, picante, taco sauce						2 tablespoons			
Refried beans or pinto beans						3/4 cup			
Kidney beans						3/4 cup			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year do you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year*. If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Broad beans (Fava beans)						3/4 cup			
Soy beans cooked from dried						¾ cup			
Other beans such as navy, white, northern, black or lima beans						3/4 cup			
Mushroom						10 raw slices or 1/2 cup cooked			
Celery						1 stalk or 1/3 cup cooked			
Beets						1/2 cup			
Broccoli						1/2 cup			
Cauliflower or Brussels sprouts						1/2 cup			
Bok Choy						1/2 cup			
Raw spinach						3/4 cup			
Cooked spinach						1/2 cup			
Turnip greens						1/2 cup			
Mustard greens, collards						1/2 cup			
Kale						1/2 cup			
Fresh garlic **(do not include garlic powder)**						1 clove			
Garlic powder						1 clove equivalent			
Red cabbage						1/2 cup			
Green cabbage, cole slaw, sauerkraut						1/2 cup			
Carrots or mixed vegetables which include carrots						1/2 cup			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods during the last year. If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Corn						1/2 cup			
Asparagus						1/2 cup			
Alfalfa sprouts including on sandwiches						1/2 cup			
Soybean sprouts						1/2 cup			
Other sprouts (not alfalfa or soybean)						1/2 cup			
Salads made with lettuce						1 medium bowl			
Salad dressings, mayonnaise, or mayonnaise type salad dressings used on sandwiches or salads						2 tablespoons			
Fakin' Bacon Bits® or Betty Crocker Bac 0's®						1 tablespoon			
French fries and fried potatoes including hash brown and in breakfast tacos						3/4 cup			
White potatoes including boiled, baked, mashed, and in potato salads						(1) or 1/2 cup			
Sweet potatoes or yams						(1) or 1/2 cup			
Cucumbers, raw						(1) or 1/2 cup			
Onions						1/2 cup			
Sweet green peppers						(1/3) or 2 tblsp			
Avocado, guacamole						(1) or 1/2 cup			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column	SMALL	MEDIUM	LARGE
Butter, margarine or other fat on vegetables, potatoes, etc. added at the table						How many pats each eating occasion?	1	2	3
Tomato juice or V-8® type Cocktail juice						6 fl. oz. glass			
Catsup						2 tablespoons			
Peanuts, peanut butter						2 tablespoons			
Pistachios or cashews						1/2 cup			
Ice cream (regular or lowfat)						1 scoop or 1/2 cup	S	M	L
Doughnuts, pastry, sweet rolls						1 piece	S	M	L
Cookies or cake (includes all types: regular, lowfat, or fat free)						1 medium piece or 3-5 cookies	S	M	L
Pumpkin pie, sweet potato pie						1 medium slice	S	M	L
Other pies						1 medium slice	S	M	L
Chocolate candy, chocolate candy bars						small bar, 1 oz.	S	M	L
Other non chocolate candies or jelly						3 pieces or 1 tablespoon			
Honey						2 teaspoons			
Biscuits, muffins (including fast food types biscuits or muffins)						1 medium piece	S	M	L
Bagels, English muffins, hamburger buns						1 medium piece	½	1	2

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?			
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE	XLARGE
Dark bread, including whole wheat, rye and pumpernickel including dark bread for sandwiches						How many slices each time?	1	2	3	
White bread, French or Italian bread, including white bread for sandwiches						How many slices each time?	1	2	3	
Corn bread, corn muffins, hushpuppies						How many pieces each time?	1	2	3	
Flour tortillas "on the side"						How many tortillas each time?	2	4	6	8
Corn tortillas "on the side"						How many tortillas each time?	2	4	6	8
Margarine on breads or rolls						How many pats each time?	1	2	3	4
Butter on breads or rolls						How many pats each time?	1	2	3	4
Cooked cereals such as oatmeal, oat bran or grits						1 medium bowl	S	M	L	
Fiber cereals like raisin bran, granola or shredded wheat						1 medium bowl	S	M	L	
Pre-sweetened cereals like frosted flakes						1 medium bowl	S	M	L	
Other cold cereals like corn flakes or Cheerios®						1 medium bowl	S	M	L	
Milk on cereal						1/2 cup				
Sugar added to cereal						1 teaspoon				

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column	SMALL	MEDIUM	LARGE
Breakfast bars, granola bars, power bars						1 medium serving			
Diet supplements (liquid or powder form) such as Ensure®; diet shakes, breakfast shakes such as instant breakfast						1 medium serving			
Pancakes or waffles						2 medium			
Cottage cheese						1/2 cup			
Regular, low fat, or fat free hard cheeses (cheddar, jack, colby, queso chihuahua, asadero and similar cheeses)						How many slices or ounces each eating occasion?	1	2	3
Cream cheese						2 tablespoons, 2 oz.			
Cheese spreads, cheese slices (regular, low fat or fat free types)						How many slices or ounces each eating occasion?	1	2	3
Yogurt and frozen yogurt (regular, low fat or fat free types)						1 cup or 8 oz. container			
Whole milk or chocolate whole milk **(not including milk on cereal)**						8 fl. oz. glass			
2% Milk (or chocolate 2% milk) **(not including milk on cereal)**						8 fl. oz. glass			
Skim milk, 1% milk or buttermilk **(not including milk on cereal)**						8 fl. oz. glass			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?			
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year*. If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE	X LARGE
Snacks like nachos with cheese, potato skins with topping						1 medium serving				
Salty snacks such as potato chips, corn chips, tortilla chips, and popcorn						2 handfuls	S	M	L	XL
Soy sauce, in cooking or added at the table						2 teaspoons				
The following spices: basil, cardamom seed, fenugreek, rosemary, sage, spearmint, or tarragon						dash for flavor				

Instruction Sheet for Meat Section

This section is about your usual eating habits in the past year for meat, poultry and fish. Common methods of cooking include pan-frying, deep-frying, broiling, baking, grilling and microwaving. Below a food item (such as hamburgers and cheeseburgers), each food item line lists a method of cooking for that particular food item.

Step 1: On each cooking method line, write the number of times a day, week, month, or year that you usually ate the food item that has been cooked in that manner.

Step 2: Don't forget serving size. Thinking in terms of all cooking methods, check either small, medium, or large for serving size.

In the example below:
- Pan fried hamburgers were eaten about twice a month
- Grilled or barbecued hamburgers were eaten about once a year
- Fast food cheese hamburgers were eaten about three times a week
- Microwaving hamburgers was another method of cooking used once a month
- Oven broiled was never or rarely used to cook hamburgers and cheeseburgers
- A medium serving was the usual serving size

DURING THE LAST YEAR

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year*. If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Hamburgers, cheeseburgers (in total, all cooking methods)						1 medium or 4 oz.		✓	
Pan-fried:			*2*						
Grilled/Barbecued:				*1*					
Oven-broiled					✓				
Fast-food		*3*							
Other *Microwaving* (specify)			*1*						

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Cooking Method Definitions:

Pan-fry: to cook meat in a preheated heavy frying pan or griddle (with or without added fat).

Grill/Barbecue: to cook the meat by placing it on a grid over coals, open fire, or ceramic briquettes heated by gas.

Oven-broil: to cook meat by placing it below the heat source such as in an oven after setting it on broil.

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Hamburgers, cheeseburgers (In total, all cooking methods)						1 medium or 4 oz.			
Pan-fried:									
Grilled/Barbecued:									
Oven-broiled									
Fast-food									
Other _____ (specify)									
Other ground beef including meat loaf or tacos						1 cup			
Beef Steaks (in total, all cooking methods)						4 oz.			
Pan-fried:									
Grilled/Barbecued:									
Oven-broiled									
Other _____ (specify)									
Beef Roast (including sandwiches)						4 ounces			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Barbecued beef (brisket or other beef cuts used for barbecue but not including hamburger or beef steaks)						4 ounces			
Cooked on a grill									
Oven baked									
Pork Chops (in total, all cooking methods)						2 chops or 4 oz.			
Pan-fried:									
Oven-broiled									
Baked/Roasted									
Other _____ (specify)									
Pork roast						4 ounces			
Bacon (in total, all cooking methods)						2 strips or pieces			
Pan-fried:									
Grilled/Barbecued:									
Oven-broiled									
Other _____ (specify)									

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Sausage (in total, all cooking methods)						2 breakfast sausage or 1 other sausage			
Pan-fried:									
Grilled/Barbecued:									
Oven-broiled									
Other _____ (specify)									
Hot Dogs or Franks (in total, all cooking methods)						2 hot dogs			
Pan-fried:									
Oven-broiled									
Grilled/Barbecued									
Other _____ (specify)									
Ham, bologna, salami, and other lunch meats						2 slices or 2 oz.			
Fried Chicken (in total, all cooking methods)						1 large piece or 2 small			
Deep fat fried/fast food									
Pan-fried									
Other _____ (specify)									

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Chicken (in total, all cooking methods						1 large piece or 2 small			
Baked/roasted									
Stewed									
Oven-broiled									
Grilled/Barbecued									
Other_____ (specify)									
Fried fish/Fish Sandwich (in total, all cooking methods)						4 oz. or 1 sandwich			
Pan-fried									
Deep fat fried/fast food									
Other _____ (specify)									
Tuna fish (all forms)						4 oz. or 1/2 cup			
Oven-broiled									
Baked									
Casserole									
Salad									
Other _____ (specify):									

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Other Fish not including fried fish or tuna fish (in total, all cooking methods)						4 oz. or 1/2 cup			
Oven-broiled									
Baked									
Casserole									
Salad									
Other _____ (specify)									
Smoked fish						2 pieces or 4 oz			
Pickled fish such as pickled herring						2 pieces or 4 oz			
Salt cured fish such as lox						2 pieces or 4 oz			
Oysters, clams, mussels						5 pieces, 1/4 cup, or 3 oz.			
Shellfish (shrimp, crab, lobster)						5 pieces, 1/4 cup, or 3 oz.			
Packaged or canned **meatless vegetarian** chili						1 cup			
Canned chili such as Hormel® or Wolf®						1 cup			
Homemade, restaurant, or other types of chili with beans or chili con carne						1 cup			

DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Beef Stew or Potpie with carrots or other vegetables						4 ounces			
Liver, including chicken livers						4 ounces			
Meat Gravies made with meat drippings						2 tablespoons			
Spaghetti, lasagna, other pasta with tomato and meat sauce						1 cup			
Pizza						2 slices			
Burritos or tacos with meat or beans						1 medium or 2 small			
Cheese dishes without tomato sauce such as macaroni and cheese						1 cup			
Pasta salad, other pasta without tomato sauce						3/4 cup			
Rice or dishes made with rice						3/4 cup			
Vegetable soups						1 medium bowl			
Other soups containing meat such as beef or chicken						1 medium bowl			
Lentil soup, pea and bean soups						1 medium bowl	S	M	L
Soup made with Miso						1 medium bowl	S	M	L
Other meatless soups, like mushroom, cup-a-soup, ramen						1 medium bowl	S	M	L

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DURING THE LAST YEAR or PRIOR TO DIAGNOSIS

FOOD ITEM	HOW MANY TIMES (*Number*)?					MEDIUM SERVING	YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items listed below? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.* If you rarely or never ate the food item, place a check mark in the rare or no column.	DAILY	WEEKLY	MONTHLY	YEARLY	RARE or NO	How does your serving size compare? Place a check mark in either the small, medium, or large column.	SMALL	MEDIUM	LARGE
Egg substitutes, Egg Beaters®, egg whites						2 egg equivalent	1	2	3
Eggs at any meal (do not include egg substitutes)						1 egg = small 2 eggs = medium			

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32. In the past year, when you ate the following items, how were they <u>usually</u> cooked?

Hamburgers or Cheeseburgers

_____ did not eat
_____ rare
_____ medium rare
_____ medium
_____ medium-well
_____ well-done
_____ very well-done
_____ don't know

Steak

_____ did not eat
_____ rare
_____ medium rare
_____ medium
_____ medium-well
_____ well-done
_____ very well-done
_____ don't know

Chicken

_____ did not eat
_____ just until done
_____ well-done
_____ very well-done
_____ don't know

Pork Chops

_____ did not eat
_____ just until done
_____ well-done
_____ very well-done
_____ don't know

Hot Dogs or Sausage (includes sausages such as breakfast links, patties, Wurst and Kielbasa)

_____ did not eat
_____ just until done
_____ well-done
_____ very well-done
_____ don't know

33. In the past year, when you ate the following items, how were they <u>usually</u> cooked?

Bacon	**Sausage**	**Gravies**
_____ did not eat	_____ did not eat	_____ did not eat
_____ just until done	_____ just until done	_____ made from meat drippings
_____ well-done/crisp	_____ well-done/crisp	_____ store bought cans
_____ charred	_____ charred	_____ store bought packets
_____ don't know	_____ don't know	_____ don't know

34. During the past year, how often did you use the fat from fried bacon in your cooking?

_____ never	_____ 2-3 times per month	_____ 3-4 times per week
_____ less than once a month	_____ once a week	_____ 5-6 times per week
_____ once a month	_____ twice per week	_____ once or more per day

35. In the summer months, how often did you eat grilled (barbecued) meats (including beef, pork, chicken, or fish)?

_____ never	_____ 2-3 times per month	_____ 3-4 times per week
_____ less than once a month	_____ once a week	_____ 5-6 times per week
_____ once a month	_____ twice per week	_____ once or more per day

36. During the remainder of the year, how often did you eat grilled (barbecued) meats (including beef, pork, chicken, or fish)?

_____ never	_____ 2-3 times per month	_____ 3-4 times per week
_____ less than once a month	_____ once a week	_____ 5-6 times per week
_____ once a month	_____ twice per week	_____ once or more per day

37. During the past year, when you had grilled or barbecued meats how often were they charred?

_____ never	_____ about 3/4 of the time
_____ about 1/4 of the time	_____ about 100% of the time
_____ about 1/2 of the time	_____ did not eat pan-fried or oven-broiled meats

38. During the past year, when you had pan-fried or oven-broiled meats how often were they well browned?

_____ never	_____ about 3/4 of the time
_____ about 1/4 of the time	_____ about 100% of the time
_____ about 1/2 of the time	_____ did not eat pan-fried or oven-broiled meats

39. Are there any foods on the list below or additional food items that were NOT LISTED on pages 15-30 that you usually ate during the last year or prior to your diagnosis?

_____ **NO** _____ **YES**

If yes, please tell us about the foods in the space below. Record the food name, the number of times you usually ate the food item(s) during the last year or prior to your diagnosis and the usual serving size.

FOOD ITEM	HOW MANY TIMES (*Number*)?				YOUR SERVING?		
How many times a day, week, month, or year did you usually eat the food items? Place a number in either the day, week, month or year column to show how often you *usually* ate these foods *during the last year.*	DAILY	WEEKLY	MONTHLY	YEARLY	SMALL	MEDIUM	LARGE

Did you eat any of the following food items? If so, record above.

- Chicken or turkey pot pies or other mixed dishes containing chicken
- Tofu either firm or soft type such as Mor-Nu Silken Tofu, bean curd
- Tofu based sour cream, tofu based cream cheese, tofu based salad dressings
- Tofu frozen yogurt and other non dairy frozen desserts such as those made with Living Lightly® or Tofutti Fruitti®
- TVP (textured vegetable protein)
- Tempeh
- Breads made with soy flour
- Soy nuts and other snacks made with soy
- Soy cheese, cheese made with tofu
- Soy milk
- Frozen entrees made with soy cheese (such as soy based veggie lasagna, burrito, and soy based pizza
- Meat substitutes made from soy, "like soy burgers, links, sausage or tofu pups" (Examples: Worthington®, Morningstar Farms® or Green Giant®)
- Metamucil® or Psyllium seeds
- Cereals or breads made with flaxseed; plain flaxseed, or flax seed oil
- Any other foods not mentioned on the questionnaire (*record information above*)

40. Have you ever taken the double strength antacid/anti-gas Mylanta II?

_____ No _____ Yes **If YES, at what age(s) did your take it?** _____

41. Have you ever taken the laxative, Milk of Magnesia (plain)?

_____ No _____ Yes **If YES, at what age(s) did your take it?** _____

42. This questionnaire was completed by: *(Check all answers that apply)*

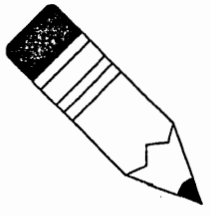
_____ Study subject
_____ Spouse/significant other
_____ Sibling/child
_____ Other (specify): _____

43. Did the person(s) who helped complete this questionnaire live with the study subject?

_____ No _____ Yes _____ Not applicable

Thank you very much for your assistance.

Please return completed questionnaire in the attached self-addressed stamped envelope.



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APPENDIX B

Visual check procedure

Status: _____ Bondy ID: _____ Chang ID: _____ Acrostic: _____ Visual Check: _____ Date: _____

Breast Study Pre-Data Entry Visual Check

#	Topic	Problem? No	Problem? Yes	Action
1	**ID & Demographic Information & Question 1-5 on Page 1**			
	Inaccurate or incomplete?			
2	**Vitamin Section--Pages 3-9**			
	Vitamin supplement frequency question incomplete?			
3	**Adjustment & Summary Questions (questions 14-22, 25- 30)-on Pages 10-13**			
	Skipped questions?			
	Incomplete or multiple answers (when not allowed)?			
4	**Food Frequency Section-Pgs 15-30**			
	Skipped pages?			
	Entire pages with check marks?			
	More than 25% of any one food category *skipped*?			
	More than 25% of any one food category *checked*?			
	> 20 portion sizes skipped?			
5	**Additional Information**			
Pg 24	Cooking methods frequency skipped			
Pg. 31	Questions 32-38 (not completed?)			
Pg 20	Butter / margarine tally >15 *per day*?			

	Fat type	Frequency D 1	W 2	M 3	Y 4	R 5	Serving Size (Pats) S 1	M 2	L 3	Total Pats per Week (Frequency X Serving Size)	Action
Pg 20	Butter, margarine or other fat on vegetables, potatoes,etc. added at the table?										
Pg 21	Margarine on bread/ rolls?										
Pg 21	Butter on bread or rolls?										
	Total Margarine *plus* Butter Pats per week										

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APPENDIX C

Edit check procedure

HHHQ Edit Check Report

- Purpose: "...to identify errors and outliers in the responses to the diet questionnaire. This then gives the investigator the opportunity to correct or exclude offending records. While error checking is obviously a component of investigators' data-management, **this program provides a standardized mechanism** so that one can be sure that the same criteria are being applied to all questionnaires. It does not, of course, preclude investigators from conducting additional scrutiny of their data. Indeed it is hoped that this will assist them in doing so." *(DIETSYS MANUAL, Chapter 14.1 Introduction to the DIETSYS Edit Checking Feature.)*

Num.	Error Description	HHHQ Warning	HHHQ Error	Action
1	Foods with miscodes / invalid coding	N/A	1 or more miscodes	Correct all miscodes
2	Miscodes / invalid coding for questions other than food frequency list	N/A	1 or more miscodes	Correct all miscodes
3	Foods skipped	10% to 15%	> 15%	Re-query all skipped items on questionnaires flagged at the "Error" level
4	Too few foods per day	Males: < 6 Females: < 5	Males: < 5 Females: < 4	Not considered but excluded if calories < 600
5	Error: Too many foods per day	N/A	> 30 food	Not considered but excluded if calories > 5000
6	Foods coded or imputed as once per time unit	70% to 75%	> 75%	Re-query all "once per's" on questionnaires flagged at the "Error" level
7	Foods coded as small serving size	85% to 99%	> 99%	Acceptable at all levels
8	Foods coded as medium serving size	94% to 99%	> 99%	Re-query skipped portion sizes only if >20 portion sizes are skipped on visual check
9	Foods coded as large serving size	85% to 99%	> 99%	Acceptable at all levels
10	Foods coded as extra large serving sizes	85% to 99%	> 99%	Acceptable at all levels
11	Questionable high frequency See attached documentation for individual and group limits for food items and food groups	1 to 2 foods; 1 or more high group frequencies	3 or more foods; No error level for groups	Re-query all questionably high food frequencies on questionnaires flagged at either the "Warning" or the "Error" level Questionably high group frequencies for milk and breads will be re-queried. All other high group frequencies are allowed
12	Questionnaire with questionable data	N/A	1 or more	All "Warnings" and "Errors" will be acted on in accordance to the above "Action" guidelines.

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