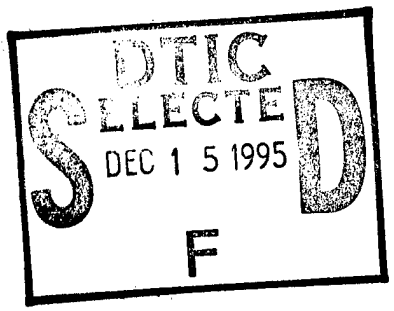

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PRINCIPAL INVESTIGATOR: Margo N. Woods

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13. ABSTRACT *(Maximum 200 words)*

Hormone replacement therapy (HRT) is being sought by 30-40% of postmenopausal women (1). Recent studies suggest that HRT should not be used by women with breast cancer or by women at increased risk for the disease since HRT increases breast cancer risk in such women by 1.5 to 3.4 fold (2-6). Currently, no safe alternative to HRT is available to treat menopausal symptoms in these women.

Our research proposal will investigate the efficacy of an alternative treatment for menopausal symptoms using a soy dietary supplement bar containing high levels of phytoestrogens. Phytoestrogens, genistein and diadzein, have recently been reported to have weak estrogen-like properties and have demonstrated binding to estrogen receptors (7-8). The low levels of reported menopausal symptoms of hot flashes and night sweats in Japanese postmenopausal women as well as the lower risk of breast cancer in Japanese women have been suggested to be due to the high consumption of phytoestrogens in soy products in this population (9a).

Identifying a substitute for HRT that alleviated the hot flashes of menopausal women but did not carry an increased risk for breast cancer would be of important clinical significance to women at increased risk for breast cancer.

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Mary N. Wood — October 25, 1995

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4. Table of Contents

5. INTRODUCTION: BACKGROUND /PURPOSE/DESIGN/RECRUITMENT

A. Introduction

Menopause,Hormone Replacement Therapy and Breast Cancer
The use of hormone replacement therapy (HRT) has increased in recent years and presently 30% of American women in early menopause receive HRT (1). While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms, there are concerns about the effect of HRT on increasing the risk of breast cancer (2-6). Two recent meta-analyses (13,14) reported an increased risk of breast cancer with estradiol products and increased risk with longer duration of use (13,15). The current research does not allow clear interpretation of the data since the dose and type of drug varied from study to study. Steinberg et al. found in their meta analysis that women on HRT with a family history of breast cancer showed an increased breast cancer risk of 3.4 (13). Colditz, et al., in the Nurses Health Study reported an increased risk of breast cancer in women currently taking estrogens but no effect in those who took them in the past (16). The current recommendations are to avoid HRT in menopausal women who are at increased risk for the breast cancer, leaving these women without a safe alternative treatment for relief of menopausal symptoms.

Serum Hormones at Menopause and Menopausal Symptoms
During the perimenopausal phase some women experience irregular menstrual cycles (17,18) and fluctuation in hormone levels. Estradiol excretion from the ovaries is decreased, which triggers a marked increase in FSH and to a lesser extent LH. Increases of these gonadotropins from the hypothalamus-pituitary axis is a standard response in the female to low levels of estrogen to induce more estrogen production in the ovaries. However, at menopause the ovaries are unable to respond to this trigger. Levels of FSH that are consistently elevated (>25 IU/liter) or FSH/LH ratio > 1 are often used to define menopause. These gonadotrophins are useful markers of relative post-menopausal estrogenation (19). At menopause, estradiol and estrone are approximately /3 to ½ lower than baseline levels normally found in the follicular phase of the cycle, in premenopausal women, and show no rise during the month when tested by multiple serum sampling.

The potential sources of estrogen compounds in menopausal women include: 1) residual excretion of estrogen from the ovaries (20) plus ovarian excretion of at least 30% of the serum androstenedione, (a precursor of estrone), and 2) androgens, produced in the adrenals and secreted into the blood (21-24), which can be converted in the adipose tissue to estrogens by the enzyme, steroid aromatase (22,25,26). In addition, alcohol intake can further stimulate the conversion of androstenedione to estrogen via induction of the steroid aromatase enzyme (27-29).

Commonly held beliefs concerning symptomatology of menopause are currently being questioned (30-32) and many of the affective disorders ascribed to menopause are found to be based on inadequate data (33). Menopausal symptoms which are cited as most problematic are "hot flashes" and "night sweats" and occur in 50-85% of women (34,35). The study by Avis, et al (37) provides a cross cultural reporting of symptoms and indicates that Canadian and American women report hot flashes and night sweats at a rate of 46 and 43%, respectively, while only 17% of Japanese women report these symptoms in natural menopause. Locke (37) reported that 9.7% of Japanese women had hot flashes and 3.6% had night sweats; in contrast, 30.9% of Canadian women reported hot flashes and 19.8% night sweats. One study investigating menopausal symptoms reported that these symptoms are inversely related to serum estrogen levels (11); those women with lower serum estrogens had higher reported symptoms. This appears to contradict the data available on Japanese women who have lower levels of estrogens and report fewer of these symptoms (37). Differences in cultural expectations have been cited as one explanation for these variations in menopausal symptoms, but there are also other possible reasons which include dietary patterns. Japanese women consume high levels of soy products which contain phytoestrogens and this might impact their hormonal status and symptomatology. The possibility that soy products can influence the sex hormone metabolism of Japanese women is of interest since they report lower menopausal symptoms and have decreased estrogenation.

Phytoestrogens

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods such as whole grains, seeds, and legumes (especially soy) and are known to have both estrogenic and anti-estrogenic properties. The two most prevalent phytoestrogens are genistein and daidzein and they appear in high concentrations in soy products. High intake of soy products has been suggested to be the contributing factor in the low incidence of breast and prostate cancer observed in Japanese women and men respectively (38). Intake of dietary soy has been shown to be inversely associated with breast cancer risk in Singapore (39). In addition to phytoestrogens (isoflavones), two other categories of compounds (flavones and lignans) have also been investigated for their estrogenic properties. Adlercreutz, et al. (40-45) have published a series of papers on populations eating diets with different intakes of fiber and compared their urinary phytoestrogens and lignan concentrations. The findings show that strict vegetarians (macrobiotics) have elevated levels of urinary phytoestrogens and lignans compared to omnivores. Japanese women had elevated urinary phytoestrogens compared to omnivores but not significantly higher lignans. Based on these studies, Adlercreutz et al. concluded that the concentrations of anti-estrogenic plant compounds in the urine are correlated with plant food consumption (40,46). They further suggested that isoflavanoids stimulate the synthesis of sex hormone binding globulin (SHBG) in the liver (47,48). Support for this comes from studies on urinary levels of phytoestrogens and lignans and plasma concentrations of SHBG in Finnish omnivores and vegetarian females (47,48). The elevated SHBG would result in a decrease in the percent of free estradiol available to bind at estrogen receptor sites. Phytoestrogens have weak estrogenic activity, as demonstrated by binding to the type I estrogen receptor (7,8), and also anti-estrogenic activity (9). The anti-estrogen properties are related to the ability of phytoestrogens to compete with estradiol, estrone and estriol for the nuclear type II receptor (9).

The estrogenic activity is determined by the affinity for the estrogen receptor and the phytoestrogens are one fiftieth to one two hundredth of that of estradiol (7). The ability of phytoestrogens to bind estrogen receptors (7,8) increase SHBG and exhibit estrogenic activity in rats as determined by vaginal cytology (49) indicates the potential of the phytoestrogens to substitute in selective ways for endogenous or exogenous estrogens. Another possible role for the phytoestrogens may be as an alternative to exogenous hormones for decreasing menopausal symptoms. This area deserves serious consideration given the risk associated with administration of exogenous estrogen in women at increased risk of breast cancer. In addition, there are also reports on the possible anticarcinogenic effects of the phytoestrogens (50,51,52).

This research seeks to find an alternative to HRT for treatment of menopausal symptoms in women at increased risk for breast cancer. Our proposed study will use women at high risk for breast cancer to investigate the effect of a dietary supplement made from soy on the frequency and intensity of menopausal symptoms of hot flashes and night sweats. We will also determine whether menopausal symptoms are correlated with endogenous estrogen hormones (E_1, E_2, E_1SO_4, FSH and androstenedione. Urinary phytoestrogens will be measured to document any correlation between phytoestrogens and symptoms and serum hormone levels, and to determine compliance with the intervention.

B. Hypothesis

The use of a soy dietary supplement bar in women at increased risk for beast cancer and reporting high menopausal symptomatology will result in a decrease in symptoms as well as alterations in endogenous hormone levels.

C. Specific Aims

1. Recruit 100 high risk menopausal women for the study who are experiencing frequent and consistent menopausal symptoms of hot flashes ($\geq$5 per daytime hours) and/or night sweats ($\geq$5 per week) to participate in an intervention study. Recruit 100 high risk menopausal women without symptoms who will act as a control group in which only baseline data will be collected.

2. Use a randomized, cross-over study design, in which high risk women with high menopausal symptoms will be given a soy dietary supplement bar or a placebo bar for 3 months, with a wash out period of 1 month, followed by the alternative dietary intervention.

3. Collect data on menopausal symptoms using a daily symptoms diary for the duration of the intervention study.

4. Collect blood samples for determination of sex hormone levels for two days at baseline, after three months of soy supplementation and after three months of placebo.

5. Collect a 24 hour urine sample for determination of phytoestrogens at baseline, after three months of soy supplementation and after three months of placebo.

6. Collect a food frequency questionnaire at baseline on all participants and 3-Day Food Records from the Intervention Group at baseline and at the end of each study phase, concurrent with the blood and urine collections.

D. Methods

1. Design and Flow Chart

This proposed dietary intervention study will use a randomized cross-over design (outlined below) to determine the effect of a soy dietary supplement bar on endogenous hormones and menopausal symptoms. (See Figure 1). A control group with few or no menopausal symptoms will be used to collect baseline data only.

FIGURE 1
Study Design and Parameters

	Baseline	Phase I	Phase II	Phase III
Time (months)		3	1	3
Intervention Group	(n=100)			
	I (50)	Supplement	Placebo	Placebo
	I (50)	Placebo	Placebo	Supplement
Controls (n=100)	C	-	-	-
Determinations				
Hormones	I,C	I	-	I
Dietary Data	I,C	I	-	I
Urinary Phyto estrogens	I,C	I	-	I
Menopausal symptoms	I,C	I	-	I

NOTE: I = Intervention; C = Controls

A cross over design is important in order to remove the effect of time on the menopausal symptoms and to determine the placebo effect on the subjective symptoms of menopause.

Menopausal symptoms have been reported to be present for as little as a few months and up to 5-6 years (18,72). Physical activity, life events, and weight changes have been reported to influence menopausal symptoms (17,73-75) and will also be monitored throughout the study.

2. Eligibility Criteria/Exclusion Criteria of Participants

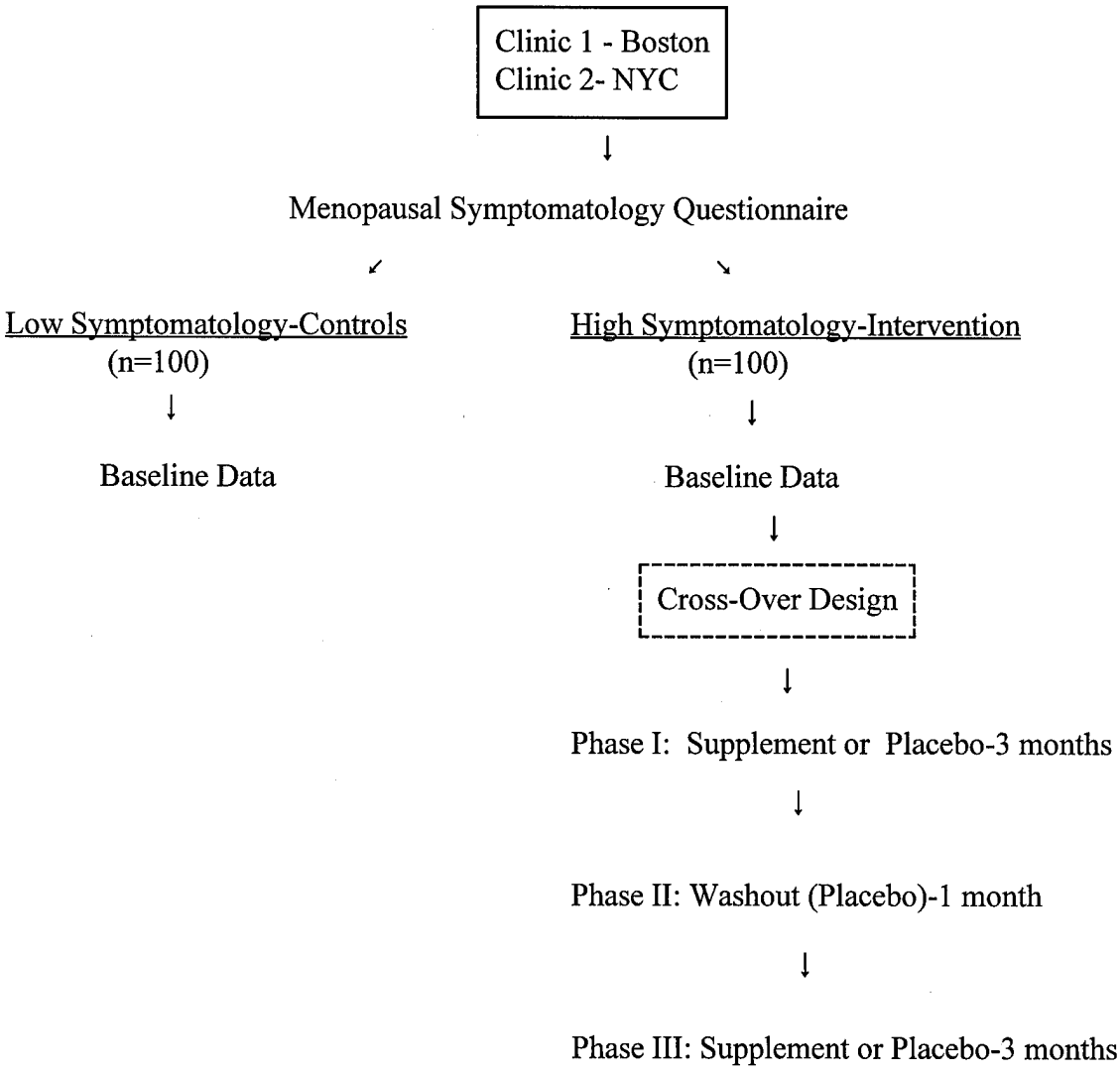
Women, ages 48-58, at high risk for breast cancer, who are post-menopausal (no periods for at least one year) are eligible for the study (Figure 2). High risk is defined as having one or more of the following characteristics: 1) mother or sister with breast cancer, 2) two or more benign breast biopsies, and 3) one breast biopsy with diagnosis of proliferative breast disease. Menopausal symptoms will be ascertained using a "Menopausal Questionnaire" and those who report $\geq$ 5 hot flashes during daytime hours and/or $\geq$5 night sweats per week will be eligible for the intervention arm of the study. Women with $\leq$2 hot flashes during daytime hours or $\leq$1 night sweats per week will be eligible for the control group. Women recruited must be within 90-120% of ideal body weight.

An extensive medical history will be taken and those women with the following characteristics will be excluded: surgical menopause (no intact ovaries); currently on hormone replacement therapy (HRT); previous use of HRT within the last 6 months; previous breast cancer; history of frequent use of antibiotics (>2 times over the last 3 years); and high intake (> 25 gm/day) of soy products.

FIGURE 2

Study Design: Randomized Cross-Over Design for a Soy Dietary Supplement Bar

Recruit: Women at high risk for breast cancer
Menopausal: ($\geq$ 1 year since last menstrual cycle) Age: 48-58 years



3. Recruitment

Two sites will be used to recruit the high risk women: Tufts New England Medical Center-Breast Health Clinic in Boston and Memorial Sloan Kettering Cancer Center- Special Surveillance Breast Program in New York City. Each site will recruit 50 high risk women with high menopausal symptomatology and 50 high risk women with low menopausal symptomatology.

a. Tufts New England Medical Center (T-NEMC)/ Breast Health Clinic

Since its formation in 1981, the New England Medical Center Breast Health Center (BHC) has been a multidisciplinary out-patient unit dedicated to the diagnosis and treatment of breast disease. The center is currently directed by Dr. Thomas Smith and is located on the 6th Floor of the Biewend Medical Building - the main New England Medical Center office building.

Recruitment for this study will be conducted via letters to current age appropriate, high risk patients at the BHC. There are currently 5,500 patient visits annually, with 3,000 registered patients and 1,200 new patients added each year. Twenty to twenty-five percent of these patients are in the appropriate age range of 48-58 years of age and 50% are estimated to be at high risk due to family history or benign biopsy. If additional participants are needed, a recruitment strategy would include advertisement through the NEMC newsletters and to the general population via the two large Boston daily newspapers.

Estimates on prevalence of menopausal symptoms are based on a number of reports on U.S. menopausal women (18,33-35,37). The data from the Massachusetts Women's Health Study, which is the largest epidemiological study of the health of middle-aged women, included over 8,000 women and was started in 1982 with data collection continuing. Results from this study indicate that 35% report having hot flashes and 30% report trouble sleeping with 11% reporting night sweats (37). This data does not quantify the frequency of these symptoms, and it is estimated that 15-20% of age-eligible, high risk women would have the intensity of symptoms needed to make them eligible for this study: $\geq$ 5 hot flashes per daytime or $\geq$ 5 night sweats per week (18). Of those eligible, we would expect to be able to recruit and retain approximately 30% into the study. High risk women with low levels of symptoms of menopause (defined above) will be eligible for the control group (N=50).

Using these assumptions, we estimate that we will be able to recruit 15 age eligible, high risk, symptom eligible women from our current clinic rosters. An additional 12 women can be recruited each year from newly enrolled patients. The accrual of subjects will continue over 2 ½ years. This predicts that almost all the Boston study population (n= 50) could be recruited from our BHC. A large cohort of low-symptom control women is also available. Included in our proposal is the enthusiastic support letter of the director of the BHC. A trained recruiter will be available at the clinic site 3 half-days a week (which represents the scheduled clinic times). Dr. Susan Sajer, a staff oncologist and member of the BHC, will be involved in setting the clinic protocol for recruitment within the BHC and OB/GYN clinics. To enhance accrual, we plan to use strategies which have been proven effective in the recruitment of women to the NSABP breast cancer prevention trial, Protocol P-1 (Tamoxifen Study). Specifically, this includes the use of our Tufts-NEMC ECOG/NSABP network of physicians, informational sessions for interested women and advertisements. We currently have a designated Cancer Prevention nurse who is familiar with our patient population, and she has successfully used the above strategies for patient recruitment.

b. Recruitment of study subjects from the Special Surveillance Breast Program of Memorial Sloan Kettering Cancer Center

Two sources of patients are available for recruitment to the study from the newly opened Breast Diagnostic and Treatment Center of Memorial Sloan Kettering Cancer Center: women attending the Special Surveillance Breast Program and women receiving continuing care after surgery for a benign breast condition by one of the six breast specialists who are members of the Breast Service, Department of Surgery.

More than 1300 women are currently registered with the Special Surveillance Breast Program (SSBP), a breast cancer screening program specifically designed to meet the needs of women at elevated risk of developing breast cancer. The level of increased risk is estimated by the extent of family history, the ages at diagnosis of relatives, and the histology of prior benign breast surgery. As of October 1993 approximately 300 women are between the ages of 48 and 58, the target age group for the proposed study. IF 20% of these women report severe menopausal symptoms and 50% agree to participate in the study, 30 women from the currently enrolled high risk patients would be recruited and retained. The accrual of subjects will continue over 2 ½ years. An average of 25 new patients have been enrolling in the program each month during 1993, potentially providing 5 to 10 additional study subjects annually from the SSBP. Women involved in our center have been very motivated and previous experience suggests that projected enrollment of 50% is reasonable. Women who are at high risk but with low levels of menopausal symptoms are readily available for the baseline control group (n=50).

Approximately 2,500 women are surgically treated for a benign breast condition by one of the six members of the Breast Service. More than 50% of these patients are between the ages of 40 and 60 with an estimated 600 postmenopausal women available for potential recruitment to the study. If severe menopausal symptoms are reported by 20% and 50% of those who are invited to participate are recruited, the required 50 study subjects could be readily recruited.

The Study Coordinator will have the responsibility of recruiting appropriate SSBP patients to the study. She will attend each session of the SSBP to review the medical records of all patients scheduled for reexamination; the baseline history of all newly enrolled patients will also be reviewed. Participation in this study will be offered to all currently enrolled women who meet the eligibility criteria at their first scheduled examination after initiation of recruitment. In addition, all new patients will be evaluated for eligibility at the time of first examination; if appropriate, study participation will be offered at that time. Women who do not schedule reexamination appointments at recommended intervals are contacted by phone and encouraged to return to the clinic. At this time all eligible women will be informed about the study and be given the opportunity to participate at the time of their follow-up examination. Once enrolled in the intervention trial, the Project Coordinator will have the responsibility of explaining the study goals, obtaining informed consent, collecting baseline and follow-up data, arranging for collection of bloods and urine specimens, instructing patients on dietary component of the study, and providing liaison with other services available at Memorial Sloan Kettering for women at high risk of breast cancer such as genetic counseling, nutrition counseling, and psychiatry.

6. BODY: STATEMENT OF WORK/PROGRESS REPORT

The Effect of A Soy Dietary Supplement on Hormone Levels and Menopausal Symptoms in Women at Increased Risk for Breast Cancer

Task 1, Preparation to start study, Months 1-4:

a. Study questionnaires will be developed, tested and printed (screening, menopausal symptoms, medical history, food records).

b. Study recruiters will be trained.

c. Data entry forms will be developed.

d. Recruitment materials will be developed.

e. Study protocols will be written

Task 2, Recruit subjects and begin study, Months 5-40:

a. Rolling recruitment will take place from, Months 5-35:

1. Letters will be sent to age eligible patients from clinic.
2. Advertisements will be placed in newspapers as needed.

b. Subjects will enter and complete study, Months 8-42:

1. Study data will be collected and entered.

2. Serum and urine samples will be collected, processed and analyzed.

3. Dietary data will be collected and entered.

Task 3, Conclusion of Study, Months 42-48:

a. Laboratory studies will be completed.

b. Statistical analyses will be performed.

c. Final report will be written.

Progress Report/Work Accomplished

After receiving notification of funding for the study, Ann LaBrode was appointed study coordinator and started to work with Drs. Woods and Senie to develop the study protocol, Manual of Operations (MOOP) and study instruments. Dr. Senie also worked with staff at her site to develop the randomization scheme and the study data entry forms using the Paradox software program.

A. Development of Study Materials:

1. Menopausal Questionnaire: Working Dr. Kronnenberg we developed a questionnaire on menopause that was tested in our clinic. It provides information on the quality of the menopausal experience and also quantifies some specific indicators. A copy of the questionnaire appears in the Appendix (Item A).

2. Medical History Form: Our standard medical history form was reviewed and revised in order to take advantage of data that is collected at the Mt. Sinai Special Surveillance Breast Program and minimize the duplication of data collection. The final form is shown in the Appendix (Item B).

3. Menopausal Symptoms Collection Instruments: Two separate instruments were developed to collect data on menopausal symptoms from the women. One is identified as the Study Log and collects data on the consumption of the dietary bars plus the number of hot flashes during waking and sleeping hours each day with each week (Sunday-Saturday) on one page with space left for notes on illness, other medications, travel, etc. that took place during the week. These factors might have an effect on reporting of symptoms or on the symptoms themselves and tracking such events may be useful in identifying confounders. A definition of hot flash is also given. This instrument is used during recruitment to identify women for the study and enroll them in the control of intervention group. It is used to verify their subjective opinion on the number of hot flashes they experience per day.

   The second instrument that was developed was the "Seven Day Daily Symptoms Diary" which requests that the participant record the time of day and intensity of the hot flash, each day for seven consecutive days during each phase of the study. This is a more detailed record of symptoms and requests documentation of each event as it happens. It will be used as the primary data source for the evaluation of the effect of soy versus a placebo bar on frequency and intensity of menopausal symptoms. These instruments are shown in the Appendix (Items C and D).

4. Food Record Booklet: A booklet was developed for the participants to record their food intake for 3 days. It also contains instructions on how to record this information with examples and prompts. This instrument is in the Appendix (Item E).

5. Manual of Operations (MOOP): Because the study was being collected at two sites, it was

important to describe each procedure in detail to facilitate standardized procedures at each site. To accomplish this a manual of operations was developed in which forms and procedures are outlined and detailed. This process is detailed and time consuming but necessary considering the different types of sites involved in the study. The MOOP is included in the Appendix (Item F). The MOOP is constantly updated and clarified as issues arise and both sites are given updated versions to maintain standard procedures. In general, pages may be substituted for earlier versions and all pages are dated. An important part of the MOOP is the data entry forms that have been developed for study data that are also present on disc, on the computers at each site. This document is the best reference for protocol questions that might arise at each site.

B. Recruitment Activities

1. Human Study Forms: Final Human Consent Forms for signature by the participants were developed and reviewed at each of the study sites. Some minor changes and clarifications were requested after the study was reviewed following the funding of the project. The current consent forms from each site are included in the Appendix (Items G and H).

2. Recruitment Material:

a.. Tufts University School of Medicine

The Boston site developed a one page recruitment flyer to be used at the Tufts-New England Medical Center Breast Health Clinic waiting rooms. Using mailing list from the Breast Health Clinic, women in the study age range were mailed material explaining the purpose and requirements of the study. Presentations on the research that supported the study goals were made by Dr. Woods to the staff at the clinic and the department of OB/GYN. Recruitment material is located in the Appendix (Items I and J).

b. Memorial Sloan-Kettering-Special Surveillance Breast Program

All the women in the Special Surveillance Breast Program are at high risk for breast cancer and therefore are an excellent group from which to recruit. Women in the appropriate age range were identified and letters of information were sent to those who were scheduled for a clinic visit within the next two months. All women in the program are seen every six months. A study coordinator was available to screen women who called the study number or who agreed to be seen at the time of their regular clinic visit.

3. Recruitment Sites:

a.. Tufts University School of Medicine

Four months after the funding of the program we had materials and advertisements available at the Tufts-New England Medical Center Breast Health Clinic. We had met with the clinic staff, presented our study rationale and design and provided them with the recruitment material for their review. Our co-investigator, Dr. Susan Sajer, who worked at the clinic had already reviewed the material. Samples of the soy bar were also provided to the physicians.

We have made contact with suburban hospitals to make presentations to their staff involved in breast care and OB/GYN, to encourage them to refer patients to our study. Study materials are available in the suburban clinics and offices. We have contacted three suburban hospitals and are planning to further develop this strategy to other small community hospitals. At the same time we are increasing our interaction with the staff at Tufts-New England Medical Center.

Six months into the study we mailed letters to age appropriate patients listed with the NEMCH Breast Health Clinic as cited above.

b.) Memorial Sloan-Kettering-Special Surveillance Breast Program

After receiving approval with outstanding merit from the Protocol Evaluation Committee of the Memorial Sloan Kettering Research Council, the study protocol was submitted to the Institutional Review Board and was approved at the meeting held on June 13, 1995. Copies of the study (#95-39) consent forms are enclosed.

During the past several months the procedures conducted at Memorial Sloan Kettering, in addition to the collaborative activities with Margo Woods and Ann LaBrode, have addressed identification of the population to be recruited to the study and arrangements for handling data and specimen collection.

The extensive files of the Special Surveillance Breast Program were searched for young women meeting the study criteria for recruitment including current age and level of risk of breast cancer. The date of last examination and subsequent appointment date were recorded. A letter of introduction was drafted to be sent to women three weeks prior to scheduled appointment date. The screening questionnaire previously developed is used at the time of follow-up phone call within the week prior to the scheduled visit. This sequence has worked well to identify women who are interested in joining the study and who meet the recruitment criteria. In addition, the project director will be advised of any new patients being examined for the first time by Drs. Heerdt or Van Zee of the Special Surveillance Breast Program, who meet the study criteria. These women will be invited to join the **Soy Study** at that time if, on review of the screening questionnaire, they meet all requirements for enrollment in the study.

After signing the appropriate consent forms, women are interviewed and complete data collection instruments at the time of enrollment at the Memorial 64th Street Breast Cancer. Blood specimens are drawn at the Breast Cancer and are carried to the Breast Cancer Laboratory in the Rockefeller Building for processing and freezing. The study subjects return 3 to 7 days later bring with them a sample of first morning urine in the container previously provided. A second blood specimen is collected. Methods for collecting the three day food records are explained during the second visit. Women with extensive symptomatology are advised of their recruitment to the intervention component of the project and are provided in the dietary supplement bars for two weeks. Phone contact is maintained weekly with subjects enrolled in the intervention and additional packages are sent by Fedex

each two weeks.

4. Current Recruitment Status:

a.. Tufts School of Medicine

We began recruiting for the study February 1995, 5 months into the study, however, due to problems with the soy dietary bar we had to put these activities on hold until these issues were resolved. See the section below for details. As of August 1995 we had received soy dietary bars and placebos that met specifications and could proceed with recruitment. We currently have five women in the study consuming the dietary bars and following the study protocol. Our original recruitment plan has not been able to proceed which would have recruited 1.4 women per month for 7 months of the first year or 10 women at the end of year one. An equal number of women with low level of symptoms would also be recruited to collected baseline data only. We have not been actively recruiting this population because we are more confident in obtaining these women and have put our effort into resolving the issue of acceptability of the dietary soy bar.

b. Memorial Sloan-Kettering-Special Surveillance Breast Program

Four women have been recruited from the Special Surveillance Breast Program into the Menopausal Symptoms Study and are currently in Phase I. Letters continue to be sent out to clinic patients prior to their scheduled visits. In addition, 250 new women are added to the clinic roster each year and therefore these new women are also contacted.

5. Projected Recruitment Plans

a.. Tufts School of Medicine

The original recruitment plan was to recruit 100 women in 35 months while the adjusted plan is to recruit 95 women in 30 months or 3 women per month. More accurately this entails recruiting 1.5 women into the high symptom-dietary intervention group per month and 1.7 women per month into the control group for just baseline data. With our current recruitment plan of contacts in the suburban hospitals we believe we can meet this slightly accelerated plan. In addition we have also begun general advertising in the Boston Globe and suburban newspapers. We currently have over 25 women going through the screening process for recruitment into the study from responses to the newspaper advertisements alone.

Community based recruitment efforts are currently underway and include participation in community outreach programs, increased media attention, contact with local Breast Cancer and Public Health organizations, research seminars, and participation in local Health Fairs. These efforts are described below in more detail.

Community Outreach Programs: Most of the suburban hospitals in the greater Boston area

have recently initiated broad based community outreach programs. These programs include health awareness programs for the general public, newsletters and extensive support networks for breast cancer patients and families. We have contacted a number of these community outreach programs and are coordinating our recruitment efforts with these programs. Presentations have been made at Emerson Hospital in Concord, MA and at a Breast Cancer Awareness Program sponsored by The Cancer Care Center at Southwood Community Hospital in Norfolk, MA.

Increased Media Attention: Articles about the study have appeared in the Tufts University Diet and Nutrition Letter (February 1995) and in the Tufts University School of Medicine Deans Rounds (Spring 1995). We are currently preparing articles for release in local newspapers and have contacted television networks for spots on local news broadcasts. Segments about our research have been aired on Channel 25 - The News at 10:00 and arrangements are currently being made with other local stations. Press releases about the study have been sent to local radio stations requesting that public service announcements been made about the study. Advertisements have now been placed in local newspapers, and notices are currently appearing in the Health/Science section of the Boston Globe. These efforts will continue.

Breast Cancer and Public Health Organizations: Local public health and breast cancer organizations have been contacted and informed about our recruitment efforts. A presentation about the study was given at the Mass Department of Public Health Women's Health Breakfast Series and the study was highlighted in the last newsletter from the Department of Public Health. We are currently working with The Massachusetts Breast Cancer Coalition and local Breast Cancer Support organizations since first degree relatives of breast cancer patients are part of the population being recruited for this study. Study representatives have attended local Breast Cancer Fundraising Efforts including the Walk for Breast Cancer held this fall in Boston. Study representatives will continue to be visible at local Breast Cancer events.

Research Seminars and Presentations: Several research seminars and presentations have been made to inform the medical and research community about our study. We are currently contacting local business's and health care organizations to organize future talks. A research seminar has been scheduled for November 7, 1995 at the United States Army Research and Development Command at Natick, Massachusetts. It is our hope that local researchers will be instrumental in passing information about the study to friends, families and the general public.

Health Fairs: We are planning to attend local Health Fairs and have designed materials to be set up and distributed at a booth. We are contacting the organizers of these events to obtain dates and reserve space.

b. Memorial Sloan-Kettering Cancer Center- Special Surveillance Breast Program

The current recruitment plan appears to be working and response has been adequate. Personnel hired and trained this summer have returned to graduate studies and a new recruiter is being trained to continue the study screening, recruitment and study protocol. Dr. Woods and Ms. LaBrode will visit the Memorial Sloan-Kettering in November to review protocols and quality control procedures and check for the consistency between the two sites.

C. Development of the Soy Dietary Supplement Bar

Prior to the submission of the grant we had worked with Standard Hospital Supplies (SHS) to develop a soy dietary supplement bar that met the criteria for the study by containing 40 mg of phytoestrogens in two dietary bars which would be consumed each day. The development of the placebo bar was not complete but was considered by SHS to be a routine production issue in which casein protein would be substituted for the soy protein. SHS also discussed doing shelf life studies on the supplement bar.

With the funding of the grant we went forward our production plans and schedule to develop the soy dietary supplement bars. However, due to some delays in revising the consent forms the dietary bars were stored for two months in our unit prior to recruitment. During recruitment presentations we would bring the dietary bars so prospective participants could determine their acceptability. It was during this process we discovered that the bars changed drastically during storage and resulted in the consistency going from a soft nougat like texture to taffy to hardened taffy. This was an unacceptable product and all attention was given to the reformulation of the dietary supplement bar.

Evaluation of the problems and the possible solutions led us to contact Protein Technologies, International(PTI) as an alternate source of the soy dietary supplement bars. They already had a chocolate flavored bar that met our expectation of phystoestrogen content. A placebo had not been formulated or tested by them, however. After considerable discussion with both suppliers we decided to use PTI as the supplier.

PTI agreed to produce the product without the chocolate covering for our study and to package it to meet our requirements. They assured us that an acceptable and indistinguishable placebo could be produced within two months. We have now received tasty, soft and acceptable soy and placebo bars from PTI for the study with 4 month shelf-life studies indicating no deterioration in the quality of the placebo product. They had previously tested the 1 year shelf life of their soy dietary supplement bar. A production schedule has been worked out with PTI to assure an ongoing supply of soy and placebo bars for the duration of the study. The nutritional assessment of the soy and placebo dietary supplement bars is presented in the Appendix (Item K).

D. Labeling and Distribution of the Dietary Supplement Bars

Supplement bars are delivered to the Tufts, Nutrition Unit were they are checked for quantity and quality. A labeling procedure has been developed for the study that is used to package the participant's supply of bars for Phase I and Phase III. This labeling and packaging is carried out by

a research associate who has access to the identification of the randomization code numbers for the participants (regarding which bar, soy or placebo, they are to receive for the Phase I and Phase III periods). No other study personnel has access to this information. This procedure has been used in the past in other studies using placebos and blinding of participants and study personnel. The bars are packaged for each individual randomized code and are ready to be supplied to either of the two sites as participants are randomized into the trial. The individual sites supply the participants with bars for only one phase at a time. At Memorial Sloan-Kettering, the bars may be mailed to the participants since most use public transportation to the clinic site and the bars may be too heavy. Subjects are asked to return any unused bars from each phase. An extra two week supply may be given to ensure availability in case the clinic visits are delayed at the end of each phase. All study personnel that are involved in recruiting or interaction with the participants in any way are blinded to the identification of the supplement bars which are indistinguishable in appearance.

E. Data Collection

1. Randomization procedures:
   See page 6 of the MOOP in the Appendix.

2. Data entry forms
   Programs have been written so that all data forms are entered on Paradox. Forms will be entered at each clinic side and standard data sets will be generated from all forms at each clinic. The data analysis will be conducted starting at the end of year 3.

F. Training of Study Personnel

Personnel at Memorial Sloan-Kettering have been trained by staff from Tufts and Dr. Senie. Certification procedures are in place for training of participants in the recording of the food record and the documentation of the returned record. In addition personnel have visited at Tufts to observe and practice the collection, storage and handling of blood and urine samples and training subjects on all the study instruments. Once a year visits between the two sites are being scheduled to maintain vigilance on quality control procedures. Previous experience with studies conducted at multiple sites has made us aware of the issues that need to be addressed.

7. CONCLUSIONS: PLANS FOR THE COMING YEAR

The current data on phytoestrogens continues to look provocative regarding its potential as a modulator of endogenous estrogen status and an anticarcinogenic agent.

We have had two national medical news teams interview myself and my colleagues on our studies involving phytoestrogens. We have received numerous phone calls from across the country, however, recruiting for our study is limited to the Boston or NYC areas. We are pleased with the palatability of the dietary supplement bars and the efficient plan of the protocol. We expect to meet our recruitment schedule and obtain high quality data on menopausal symptoms, hormones and dietary intake for our data analysis.

Identifying a substitute for HRT that alleviated the hot flashes of menopausal women but did not carry an increased risk for breast cancer would be of clinical significance to women at increased risk for breast cancer. In addition, the study would supply data on the effect of phytoestrogens on endogenous hormone levels that might indicate their possible role in decreasing risk for breast cancer.

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9. APPENDIX

A. Menopausal Questionnaire
B. Medical History Form
C. Study Log (of menopausal symptoms)
D. Seven Day Daily Symptoms Diary
E. Food Record Booklet
F. Manual Of Operations (MOOP)
G. Consent Form - Tufts
H. Consent Form - Sloan Kettering
I. Recruitment Material - Tufts
J. Recruitment Material - Sloan Kettering
K. Protein Technology, International (PTI) Supplement Bar Nutrient Content

A. MENOPAUSAL QUESTIONNAIRE

MEDICAL HISTORY QUESTIONNAIRE
Menopausal Symptoms Study (MSS)

Please answer all questions by filling in the blanks or writing in the information requested. If you need extra room use the back of the form for additional remarks. All information is strictly confidential and will be used only for medical statistical purposes

____Boston

1. Today's Date: __ __/__ __/__ __ ____New York City
   M M D D Y Y
2. Hospital ID: __ __ __ __ __ __ __ __ __ Study ID __ __ __
3. Name _____
4. Address _____

   _____
5. Zip Code: __ __ __ __ __
6. Telephone (Day) (____)_____
7. Telephone (Eve) (____)_____..
8. Birthdate: __ __/__ __/__ __
   M M D D Y Y
9. Age _____
10. Height _____ (feet, inches)
11. Weight _____ (lbs)
12. Marital Status S M W D
13. Country of Birth ___United States ___South American ___Caribbean
    ___Other, Name:_____
14. Race ____Caucasian ____African-American ____Hispanic
    _____ Asian _____Other
15. Education Grades Completed (1-12)_____ College (years)_____
    Post-college (years) _____
16. In Case of emergency call:_____ Phone _____

Pregnancy History

17. Have you ever been pregnant? ____No ____Yes
    If Yes: a. How many times have you been pregnant? _____
    b. What was your age when you were first pregnant? _____
    c. What was your age when your first child was born? _____
    d. How many pregnancies were not completed? _____
    Number of miscarriages _____
    Number of abortions _____
    e. What was your age at your last pregnancy? _____

18. Did you breast feed any of your children? _____No ____Yes
    If yes:
    a. Number of children breastfed _____
    b. Total number of months of breast feeding _____

Menstrual History

19. At what age did your menstrual periods begin? _____

20. Have you ever had irregular menstrual cycles? ____ No ____ Yes
    If yes:
    a. How often did you have irregular menstrual cycles?
    ____ rarely
    ____ consistently or often
    ____ just before menopause
    ____ Other: _____
    Explain:

21. Have you ever had heavy menstrual flow
    or severe pain with menstruation? _____ No _____ Yes

22. Have you ever had a positive PAP smear? _____ No _____ Yes
    If yes:
    a. How many times? _____
    b. Was this within the last five years _____ No _____ Yes

23. How frequent were your menstrual periods? _____ days
    (Length in days from start of one cycles to start of next cycle)

Premenopausal History Hormone Use

24. Did you ever use birth control pills?: _____ No _____Yes
    If yes: a. How old were you ? _____
    b. How many months did you use them for? _____ months
    Exact dates used if known: _____ to _____
    MM/YY MM/YY
    c. What type of birth control pills did you use?
    Please check
    ___ Estrogen only
    ___ Estrogen and progesterone
    ___ Progesterone only
    ___ Not sure
    ___ Other: _____

25. Have you used any other hormone medications prior to menopause such as other pills, patches, creams or suppositories?
    Please check.
    ____ Androgens
    ____ Hormone Patch
    ____ Hormone cream
    ____ Hormone suppositories
    ____ Not sure
    ____ Other: _____

26. Did you ever use any fertility medications? _____ No _____ Yes
    If yes: Which one did you use? Please check.
    ____ DES
    ____ Not sure
    ____ Other: _____

Menopausal History

27. Have you had a period during the last year? _____ No _____Yes ____Uncertain

28. When was your your last menstrual period? ___ ___/___ ___
    MM YY

29. What was your age when you entered menopause? _____

30. Was your menopause?
    ___ Natural
    ___ Surgical/hysterectomy **(Go to 30a)**
    ___ Other_____

    a. If surgical, did it include removal of ovaries? ____No ____Yes
    If Yes:
    ____ one ovary
    ____ both ovaries
    ____ don't know

History of Menopausal Hormone Use

31. Are you currently taking hormones? _____ No _____ Yes
    If yes: Which one? Please Check.
    ___ Estrogen and progesterone
    ___ Estrogen only
    ___ Progesterone only
    ___ Not sure
    ___ Other: _____

32. Have you taken hormones in the past during menopause? _____ No _____ Yes
    If yes: Which one(s)? Please Check.
    ___ Estrogen and progesterone
    ___ Estrogen only
    ___ Progesterone only
    ___ Not sure
    ___ Other: _____
    _____

    b. For how long did you take the hormones? _____ months
    _____ years

    c. When did you stop taking the hormones? _____ months ago
    _____ years ago

    Dates taken if known: _____ to _____

History of Breast Problems

33. Have you ever had a mammogram? _____ No _____ Yes
    If yes: When was the last one? _____

34. Have you ever had breast cancer? _____No _____ Yes
    If yes: What was the date of the diagnosis? _____

35. Do you have a history of <u>breast disease</u>? _____ No _____ Yes
    If Yes: What type? Please check.
    ___ fibrocystic disease
    ___breast cysts
    ___ lobular carcinoma in situ (LCIS)
    ___ atypical proliferative/ breast disease
    ___ Other: _____
    _____

36. Have you ever had a breast aspiration, breast biopsy or breast surgery? ____ No ____ Yes

If Yes: a. How many breast aspirations and/or biopsies have you had? _____

b. For each biopsy (bx) or aspiration (asp), please give the year, the breast affected (l = left, r = right), the type of treatment, the findings of the treatment, and the place performed.

Year	Breast	bx or asp	Findings (normal, abnormal, etc)	Place Performed
19 __ __	__l __r	__bx __asp		
19 __ __	__l __r	__bx __asp		
19 __ __	__l __r	__bx __asp		

Personal Medical History

37.

DO YOU HAVE A HISTORY OF:	NO	YES	IF YES, PLEASE EXPLAIN
High blood pressure			
Heart Disease			
Diabetes Mellitus			
Pancreatic Disease			
Liver Disease			
Bleeding Disorder			
Hyperlipidemia (high fats in blood)			
Kidney disease			
Small Bowel Disease			
Atrophic Gastritis			
Cancer			Type:
Prior radiation to the chest or breast			
Thyroid Disease			
Urinary tract infection			Number of times in last 5 years_____
Other			Explain:

38. What was the date of your last physical exam? _____

a. Who is your primary care physician? _____
(please give address and phone if known) _____
_____

39. Please list all operations and surgeries you have had?

Type of operation:	Date of operation:

40. Please list all medications you are currently taken, indicate the condition being treated and the date you started taking the medication,

Medication	Condition being treated	Date Medication Started

41. Please list all allergies (i.e. medications, foods, other)

42. Has your weight changed more than 10 lbs in the last two years?_____ No _____ Yes
If Yes, explain:

Family History of Cancer

43. To the best of your knowledge, have any of your blood relatives (living or dead) ever had cancer? ____No ____Yes

If yes, please fill in the table below using the key at the bottom of the page:

Note: Please list separately each cancer for each relative. If breast cancer, please indicate if <u>both</u> breasts were affected. (Please see the example given in the shaded area)

Relative	Type of Cancer	Age at Diagnosis	Were both breasts affected?
6	**Breast Cancer**	**45**	**No**

1 = mother
2 = sister
3 = daughter
4 = maternal grandmother
5 = maternal grandfather
6 = maternal aunt
7 = maternal uncle
8 = maternal first cousin
9 = other (maternal)
10 = other (maternal)
11 = father
12 = brother
13 = son
14 = paternal grandmother
15 = paternal grandfather
16 = paternal aunt
17 = paternal uncle
18 = paternal first cousin
19 = other (paternal)
20 = other (paternal)

(Maternal = Mother's side of family Paternal = Father's side of family)

Smoking and Alcohol History

44. Are you currently a smoker? No _____ Yes _____
If Yes:
How many cigarettes do you smoke each day? _____

45. Have you smoked a total of 100 cigarettes in your lifetime ? No _____ Yes _____
If Yes:

a. How old were you when you began to smoke cigarettes? _____ years

b. How old were you when you last smoked cigarettes ? _____ years

c. How many cigarettes did you usually smoke each day? _____

46. Have you ever lived in the same house with a smoker? No _____ Yes _____
If yes:
For how many years did you live with a smoker? _____

47. Do you drink one or more alcoholic beverages per week? No _____ Yes_____
If yes:

a. How many alcoholic drinks do you have per week? _____

b. When you choose an alcoholic beverage what do you usually choose?
(Check all that apply)

___ Beer
___ Wine
___ Spritzer
___ Hard liquor (scotch, bourbon, whiskey, gin, etc.)

Exercise History

48. During the past year what was your average time per week in minutes spent at each of the following activities?

Activities	**Minutes per week**
Walking or hiking outdoors (include walking to work)	
Jogging (slower than 10 minutes/mile)	
Running (10 minutes/mile or faster)	
Bicycling or stationary bicycle	
Cross country ski machine	
Swimming	
Tennis/Squash or Racquetball	
Aerobics/Dance Class	
Calisthenics	
Weight Lifting	
Other exercise (please specify):	

B. MEDICAL HISTORY FORM

Menopausal Symptoms Questionnaire

I.D. _____ Clinic: ____ Boston
Date: _____ ____ New York

Please indicate whether you have experienced any of the following symptoms in the **past month** . If yes, check the Frequency, circle the **Usual Intensity** and then if the Usual Intensity varies check the Intensity Varies Column. For example, if during the last month you had joint pain once or twice a week you would check the Weekly column and leave the Daily and Monthly columns blank. Then you would circle the Usual Intensity Mild (1), Moderate (3) or Severe (5) and if the usual intensity varies check the Intensity Varies column.

EXAMPLE:		Frequency (Check One)			Usual Intensity (Circle one)			Intensity
		Daily	Weekly	Monthly	Mild	Moderate	Severe	Varies
1. Joint pain	__No √ Yes	____	√	____	1	③	5	√

		Frequency (Check One)			Usual Intensity (Circle one)			Intensity
		Daily	Weekly	Monthly	Mild	Moderate	Severe	Varies
1. Hot flashes/flushes	__No __Yes	____	____	____	1	3	5	____
2. Increased heart rate (palpitations) when you are having hot flashes	__No __Yes	____	____	____	1	3	5	____
3. Increased heart rate (palpitations) when not having hot flashes	__No __Yes	____	____	____	1	3	5	____
4. Trouble sleeping for reasons other than hot flashes	__No __Yes	____	____	____	1	3	5	____
5. Headaches or migraines that disrupt your normal activity	__No __Yes	____	____	____	1	3	5	____
6. Muscle aches, pains or stiffness preventing mobility	__No __Yes	____	____	____	1	3	5	____
7. Joint pain	__No __Yes	____	____	____	1	3	5	____
8. Vaginal dryness	__No __Yes	____	____	____	1	3	5	____
9. Increased frequency of urination	__No __Yes	____	____	____	1	3	5	____
10. Urinary leakage when coughing, sneezing or during orgasm	__No __Yes	____	____	____	1	3	5	____
11.Sudden/unexplained mood swings	__No __Yes	____	____	____	1	3	5	____
12. Periods of unexplained anxiety	__No __Yes	____	____	____	1	3	5	____
13. Periods of unexplained fatigue	__No __Yes	____	____	____	1	3	5	____
14. Difficulty remembering things	__No __Yes	____	____	____	1	3	5	____
15. Fluid retention: Bloating	__No __Yes	____	____	____	1	3	5	____
16. Indigestion	__No __Yes	____	____	____	1	3	5	____

17. Other _____ __No __Yes ____ ____ ____ 1 3 5 ___

Please answer the following questions based on what you have experienced during the last month.

18. Do you usually sweat during a hot flash? ____ No ____Yes

19. Is there anything that has made your hot flashes more likely to occur? ____ No ____Yes
If yes, explain:

20. Is there anything that has made your hot flashes less likely to occur? ____ No ____Yes
If yes, explain:

21. Have you experienced any bleeding or spotting? ____ No ____Yes
If yes, explain

22. Has your pattern of hot flashes changed? ____ No ____Yes
If yes, explain

23. Have you had any changes in medical conditions ? ____ No ____Yes
If yes, explain

24. Have you had any changes in medications that you are taking ? ____ No ____Yes
If yes, explain

25. Have you taken any antibiotics? ____ No ____Yes
If yes, explain

26. Have you experienced any of the following during the **past month**? If yes please indicate when.

	No	Yes	When
A. Illness of Relative	___	___	_____
B. Death of Family Member	___	___	_____
C. Death of Close Friend	___	___	_____
D. Divorce	___	___	_____
E. Major Vacation	___	___	_____
F. Personal Illness	___	___	_____
G. Loss of Job	___	___	_____
H. Moving	___	___	_____

27. Have you experienced any other significant life events recently? No___ Yes___
If yes, explain:

28. In the **last month** have you tried any alternative therapies (in addition to regular medical care or the study requirements) to help you with your menopausal symptoms or other medical problems?

Therapies	No	Yes	Type/Kind	For which symptom or problem did you try the therapy?
A. Herbal teas	___	___	_____	_____
B. Relaxation	___	___	_____	_____
C. Meditation	___	___	_____	_____
D. Chiropractic	___	___	_____	_____
E. Acupuncture	___	___	_____	_____
F. Change in Diet	___	___	_____	_____
G. Vitamins/ Supplements	___	___	_____	_____
H. Other	___	___	_____	_____
			_____	_____
			_____	_____

C. STUDY LOG (of menopausal symptoms)

Study Log

Study I.D.#: _ _ _

Dates: _ _-_ _-_ _ to _ _-_ _-_ _
MM DD YY MM DD YY

Clinic:(Circle) Boston New York City

INSTRUCTIONS FOR COMPLETION OF YOUR STUDY LOG

This diary will collect daily information on:

1. Day of week and date
2. Number of hot flashes experienced during daytime (waking hours) and nighttime (sleeping hours)
3. The number of dietary supplement bars consumed each day.
4. Notes on medications, illness, travel etc.

Definition of hot flash: We are considering the terms hot flash, hot flush, and night sweats to be the same. These are typically sudden episodes of feeling warm, flushing, and/or sweating. These events can take place during the day or night and we are tracking the daytime hot flashes separately from the nighttime hot flashes.

NUMBER OF HOT FLASHES

Record the total number of hot flashes you have each day.

Before you go to sleep, write the number of hot flashes you had during the day in the daytime column. Write 0 if you did not have any hot flashes that day.

When you get up in the morning, record the number of hot flashes you had between the time you went to sleep and the time you woke up in the morning. Write 0 if you did not have any hot flashes during the night.

EXAMPLE

On Sunday January 1, 1995 Jane Smith had three hot flashes during the day before she went to bed and no hot flashes between the time she went to sleep Monday night and woke up Tuesday morning. Also on Monday Jane ate only 1 of the dietary supplement bars.

On Monday January 2, 1995 Jane did not have any hot flashes during the day, but after she went to sleep she had four hot flashes during the night. On Monday Jane ate both of the dietary supplement bars.

Please refer to the sample on the next page to see how this information would be recorded.

SAMPLE

WEEK OF 1/1/95 TO1/7/95				
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday	1/1	3	0	0 (1) 2
Monday	1/2	0	4	0 1 (2)
Tuesday	1/3			0 1 2
Wed	1/4			0 1 2
Thursday	1/5			0 1 2
Friday	1/6			0 1 2
Saturday	1/7			0 1 2

NOTES:

On New Years Day I was out all day and forget to eat my second soy bar.

WEEK OF		TO		
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday				0 1 2
Monday				0 1 2
Tuesday				0 1 2
Wed				0 1 2
Thursday				0 1 2
Friday				0 1 2
Saturday				0 1 2

NOTES:

WEEK OF		TO		
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday				0 1 2
Monday				0 1 2
Tuesday				0 1 2
Wed				0 1 2
Thursday				0 1 2
Friday				0 1 2
Saturday				0 1 2
NOTES:				

WEEK OF		TO		
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday				0 1 2
Monday				0 1 2
Tuesday				0 1 2
Wed				0 1 2
Thursday				0 1 2
Friday				0 1 2
Saturday				0 1 2

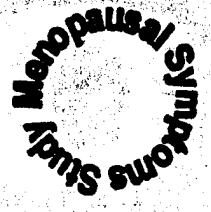
NOTES:

WEEK OF		TO		
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday				0 1 2
Monday				0 1 2
Tuesday				0 1 2
Wed				0 1 2
Thursday				0 1 2
Friday				0 1 2
Saturday				0 1 2
NOTES:				

WEEK OF		TO		
		Number of Hot Flashes		Dietary Bars Taken (Circle)
DAY	DATE	Waking Hours	Sleeping Hours	
Sunday				0 1 2
Monday				0 1 2
Tuesday				0 1 2
Thursday				0 1 2
Wed				0 1 2
Friday				0 1 2
Saturday				0 1 2

NOTES:

D. SEVEN DAY DAILY SYMPTOMS DIARY



Seven Day Daily Symptoms Diary

Study I.D.#: _ _ _

Dates: _ _-_ _-_ _ to _ _-_ _-_ _
MM DD YY MM DD YY

Clinic:(Circle) Boston New York City

Phase: (Circle) S B I II III

Instructions for Completion of Your Seven Day Daily Symptoms Diary

This diary will collect daily information on the:

- Number of dietary supplement bars consumed each day
- Date and time of all hot flashes that occur during a specified seven day period.
- Intensity of each hot flash
- Notes on medications, illness, travel, etc.

This booklet contains 1 double sided page for each day. Please start a new page for each day and record the date at the top of the page.

Number of dietary supplement bars eaten:
Each day circle the number of dietary supplement bars eaten (0 1 2).

Definition of hot flashes: We are considering the terms hot flash, hot flush, and night sweats to be the same. These are typically sudden episodes of feeling warm, flushing, and/or sweating. These events can take place during the day or night.

Number of Hot Flashes: Record the time of each individual hot flash when it occurs or as soon as possible afterwards.

Hot Flash Intensity: We would also like you to pay attention to the severity or intensity of your hot flashes and to rate them on a scale of 1 to 5:

1=Mild	a slight feeling of warmth with little or no perspiration
3=Moderate	warmer than a mild hot flash, definitely noticeable and producing obvious sensible perspiration
5=Severe	feeling intensely hot, with profuse perspiration, perhaps an unsettling feeling or feeling momentarily disrupted or debilitated

Notes: Use the Notes section daily to record circumstances that may effect the number or severity of your hot flashes. For example, if you feel ill or stressed, take medications, experience conditions that are unusually hot or cold, etc.

Day 1	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 1 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 2	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 2 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 3	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 3 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 4	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 4 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 5	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 5 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 6		Date:		
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 6 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

Day 7	Date:			
Dietary Supplements taken: 0 1 2 (Circle one)				
Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
1		1	3	5
2		1	3	5
3		1	3	5
4		1	3	5
5		1	3	5
6		1	3	5
7		1	3	5
8		1	3	5
9		1	3	5
10		1	3	5
11		1	3	5
12		1	3	5
13		1	3	5
14		1	3	5

Day 7 (continued)

Hot Flash	TIME Indicate AM/PM	Intensity (Circle one) Mild	Moderate	Severe
15		1	3	5
16		1	3	5
17		1	3	5
18		1	3	5
19		1	3	5
20		1	3	5
21		1	3	5
22		1	3	5
23		1	3	5
24		1	3	5

Notes:

E. FOOD RECORD BOOKLET

FOOD RECORD

Date of Intake: __ __ - __ __ - __ __
m m d d y y

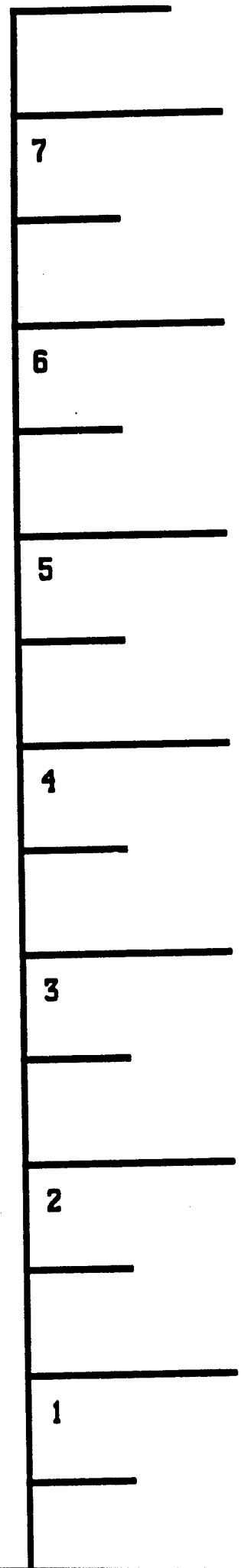
Participant's ID#: ___ ___ ___

Participant's Sex: M F (Circle)

Birth Date: __ __ - __ __ - __ __
m m d d y y

Interviewer's ID#: ___ ___ ___

Clinic: (Circle)		**Boston**		**New York**	
Phase: (Circle)	**0**	**I**	**II**	**III**	


7
6
5
4
3
2
1

Circle Day: Su M T W TH Fr Sa **Date:** __ __-__ __-__ __

SAMPLE DESCRIPTION

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION	Amount	TNDC
1	12 P	3	S	Pear. Fresh. 3" diameter w/peel	1 ea.	
2						
3	3 P	3	S	Coke. regular. no ice	12 oz.	
4				Granola Bar. Nature Valley. Cinnamon	1.5 oz.	
5						
6	6 P	1	D	Lasagna(recipe)	1/8 recipe	
7				ground beef. regular. fat	1 lb.	
8				3" onion	1 ea.	
9				clove. garlic	1 ea.	
10				16 oz. can tomatoes	1 ea.	
11				3 oz. can tomato paste	1 ea.	
12				basil	2 tsp.	
13				salt	1 tsp.	
14				lasagna. noodles. dry	1 lb.	
15				Mazola Corn oil	1 TB	
16				eggs. large	2 ea.	
17				Ricotta skim milk cheese	1 lb.	
18				Parmesan cheese. dry. grated	3/4 cup	
19				Parsley leaves. dried	2 TB	
20				Mozzarella. cheese. part skim	1 lb.	
21				Tea. herbal	1 cup	
22				Graham crackers. plain. Nabisco. 5x21/2	2 ea.	

Menopausal Symptoms Study

GUIDELINES FOR KEEPING A FOOD RECORD

Guidelines For Keeping A Food Record

1. 3 Day Food Record: Start your food record at midnight (12:01 a.m.) on day one and keep it until midnight (12:00 p.m.) on the last day. Include one weekend day.

2.Record foods and beverages immediately after eating or drinking.

- use pen
- include vitamin and mineral supplements and over-the counter medications

do not change present eating habits during collection of food record

- include all meals, snacks and beverages actually consumed anytime of the day or night.
- if "diet" or other special product, copy nutrition information from label
- include type and amount of added fat, all seasonings, sauces, and condiments
- use brand name wherever possible

3. Start a new page for each day. Use as many pages as needed.

4. List only one food per line. Skip a line between each meal.

5. Time: Record the time to the nearest hour and whether meal was consumed in A.M. or P.M.

6. Place: Indicate where food was prepared:

- Home = 1; Restaurant = 2, to indicate an expensive restaurant, use Ex and for an inexpensive restaurant, use Inex.

Other = 3, indicate whether fast food, day care, friend's, delicatessen or cafeteria.

7. Meal: Indicate: Breakfast = B, Lunch = L, Dinner = D, Snack = S.

8. Describing Amounts:

- Measure all liquids using a clear measuring cup and record in cups. (Example: milk, 2% 3/4 C.)
- If scales are not available, measure the portion consumed using tablespoons (TB), teaspoons (tsp.), cups (C), inches (in), or list the number of small items (Example: 15 raisins).
- When using your measuring cup to measure solids, report the amount in cups, not ounces. (Example: 1 cup cereal).
- If describing a portion in inches, use appropriate measurements, such as:
  - spherical food diameter, e.g. orange, 3" d
  - round food: diameter and height, e.g., cookies, 3" d x 1/4" ht.
  - square or rectangular food: length, width and height, e.g. brownie, 2" l x 3" w x 1/2" ht.
  - wedge food: arc, height and length, e.g. pie, 3" arc x 1" ht x 4" l or diameter of whole and proportion, (e.g.) 1/8th of 8" pie.

9.Describing foods, beverages and supplements:

Protein Foods:

Meat, Fish, and Poultry:

- cooked or raw weights, trimmed, partially trimmed or untrimmed
- with or without bone/shell
- method of preparation
- type, cut or part, grade or % fat
- light or dark poultry
- with or without skin
- oil or water packed fish

Legumes, Nuts and Seeds:

- dry or cooked weights

Eggs and Substitutes:

- size
- method of preparation

Soups:

- cream, milk (%) or water-based
- regular or chunky
- modified or regular

Fats And Oils:

- brand
- form (stick, tub, liquid)
- type (regular, light, diet, unsalted)

Milk Products:

- type or percent fat
- dairy or non-dairy
- liquid or powder

Grains And Mixtures:

- type of grain or flour
- recipe if homemade or mix
- thick or thin crust pizza

Desserts:

- single or double crust pies
- cake or yeast donut

Vegetables And Mixtures:

- cooked or raw weight
- method of preparation
- fresh, frozen or canned

Fruit And Mixtures:

- fresh, frozen, canned or dried
- cooked or raw weight
- sweetened or unsweetened

Sweets:

- description or recipe

Beverages:

Alcohol And Other Beverages:

- proof
- amount without ice
- light or regular beer
- table or dessert wine
- liquor or liqueur
- regular or diet
- with or without caffeine
- brewed or instant
- decaffeinated or herbal tea and coffee

Seasonings:

Include all -

- herbs and spices
- condiments and sauces
- meat tenderizer and MSG
- salts - regular or modified, plain or seasoned
- use measuring spoons if possible
- include all additions in cooking or at the table

Supplements And Over The Counter Medications:

- brand and complete name
- number of tablets or size of dosage taken

10. Recipes: For each recipe used

- Record no more than one ingredient per line.
- If the recipe is consumed again, indicate the new serving size in the same measurements as before.
- No cooking directions are required.
- Record total yield of recipe and amount eaten in the same measurement, or record proportion of total recipe eaten.

11 .After completing your food record, go back to the guidelines and check each item listed to make sure you have followed all the instructions.

APPOINTMENT INFORMATION:

Begin your Record on _____,
day

__ __ - __ __ - __ __ through _____,
m m d d y y day

__ __ - __ __ - __ __.
m m d d y y

Please contact me if any questions arise at:
tel. #:_____

Your next appointment is __ __ - __ __ - __ __.
m m d d y y

Are the three days recorded in this booklet typical of your usual food intake?

Circle: Yes No

If no, explain why?

_____
_____
_____
_____

GUIDELINES FOR KEEPING A FOOD RECORD

1. 3 Day Food Record: Start your food record at midnight (12:01 a.m.) on day one and keep it until midnight (12:00 p.m.) on the last day. Include one weekend day.

2.Record foods and beverages immediately after eating or drinking.

- use pen
- include vitamin and mineral supplements and over-the counter medications

do not change present eating habits during collection of food record

- include all meals, snacks and beverages actually consumed anytime of the day or night.
- if "diet" or other special product, copy nutrition information from label
- include type and amount of added fat, all seasonings, sauces, and condiments
- use brand name wherever possible

3. Start a new page for each day. Use as many pages as needed.

4. List only one food per line. Skip a line between each meal.

5. Time: Record the time to the nearest hour and whether meal was consumed in A.M. or P.M.

6. Place: Indicate where food was prepared:

- Home = 1; Restaurant = 2, to indicate an expensive restaurant, use Ex and for an inexpensive restaurant, use Inex.
- Other = 3, indicate whether fast food, day care, friend's, delicatessen or cafeteria.

GUIDELINES (continued)

7. Meal: Indicate: Breakfast = B, Lunch = L, Dinner = D, Snack = S.

8. Describing Amounts:

- Measure all liquids using a clear measuring cup and record in cups. (Example: milk, 2% 3/4 C.)
- If scales are not available, measure the portion consumed using tablespoons (TB), teaspoons (tsp), cups (C), inches (in), or list the number of small items (Example: 15 raisins).
- When using your measuring cup to measure solids, report the amount in cups, not ounces. (Example: 1 cup cereal).
- If describing a portion in inches, use appropriatr measurements, such as:
  - spherical food diameter, e.g. orange, 3" d
  - round food: diameter and height, e.g., cookies, 3" d x 1/4" ht.
  - square or rectangular food: length, width and height, e.g. brownie, 2" l x 3" w x 1/2" ht.
  - wedge food: arc, height and length, e.g. pie, 3" arc x 1" ht x 4" l or diameter of whole and proportion, (e.g.) 1/8th of 8" pie.

9.Describing foods, beverages and supplements:

PROTEIN FOODS:

Meat, Fish, and Poultry:

- cooked or raw weights, trimmed, partially trimmed or untrimmed
- with or without bone/shell
- method of preparation
- type, cut or part, grade or % fat
- light or dark poultry
- with or without skin
- oil or water packed fish

Protein (continued)

Legumes, Nuts and Seeds:

- dry or cooked weights

Eggs and Substitutes:

- size
- method of preparation

Soups:

- cream, milk (%) or water-based
- regular or chunky
- modified or regular

FATS AND OILS:

- brand
- form (stick, tub, liquid)
- type (regular, light, diet, unsalted)

MILK PRODUCTS:

- type or percent fat
- dairy or non-dairy
- liquid or powder

GRAINS AND MIXTURES:

- type of grain or flour
- recipe if homemade or mix
- thick or thin crust pizza

Desserts:

- single or double crust pies
- cake or yeast donut

VEGETABLES AND MIXTURES:

- cooked or raw weight
- method of preparation
- fresh, frozen or canned

FRUIT AND MIXTURES:

- fresh, frozen, canned or dried
- cooked or raw weight
- sweetened or unsweetened

SWEETS:

- description or recipe

BEVERAGES:

Alcohol And Other Beverages:

- proof
- amount without ice
- light or regular beer
- table or dessert wine
- liquor or liqueur
- regular or diet
- with or without caffeine
- brewed or instant
- decaffeinated or herbal tea and coffee

SEASONINGS:

Include all -

- herbs and spices
- condiments and sauces
- meat tenderizer and MSG
- salts - regular or modified, plain or seasoned
- use measuring spoons if possible
- include all additions in cooking or at the table

SUPPLEMENTS AND OVER THE COUNTER MEDICATIONS:

- brand and complete name
- number of tablets or size of dosage taken

10. Recipes: For each recipe used

- Record no more than one ingredient per line.
- If the recipe is consumed again, indicate the new serving size in the same measurements as before.
- No cooking directions are required.
- Record total yield of recipe and amount eaten in the same measurement, or record proportion of total recipe eaten.

11.After completing your food record, go back to the guidelines and check each item listed to make sure you have followed all the instructions.

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				
61				
62				
63				
64				
65				
66				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
67				
68				
69				
70				
71				
72				
73				
74				
75				
76				
77				
78				
79				
80				
81				
82				
83				
84				
85				
86				
87				
88				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
89				
90				
91				
92				
93				
94				
95				
96				
97				
98				
99				
100				
101				
102				
103				
104				
105				
106				
107				
108				
109				
110				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
111				
112				
113				
114				
115				
116				
117				
118				
119				
120				
121				
122				
123				
124				
125				
126				
127				
128				
129				
130				
131				
132				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTION
133				
134				
135				
136				
137				
138				
139				
140				
141				
142				
143				
144				
145				
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147				
148				
149				
150				
151				
152				
153				
154				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTIO
155				
156				
157				
158				
159				
160				
161				
162				
163				
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166				
167				
168				
169				
170				
171				
172				
173				
174				
175				
176				

Date: __ __-__ __-__ __

	Amount	Coder Use

Circle Day: Su M T W TH Fr Sa

	Time	Place	Meal	FOOD/BEVERAGE DESCRIPTIC
177				
178				
179				
180				
181				
182				
183				
184				
185				
186				
187				
188				
189				
190				
191				
192				
193				
194				
195				
196				
197				
198				

Date: __ __-__ __-__ __

	Amount	Coder Use

NOTES

TO BE COMPLETED BY INTERVIEWER:

Interviewer's ID#: ___ ___ ___

Intake was: (Circle one)

- 1 Typical
- 2 Considerably more than usual
- 3 Considerably less than usual

Information was: (Circle one)

- 1R
- 2UTR
- 3UR

Intake day/s: 1 Sunday 4 Wednesday 6 Friday
2 Monday 5 Thursday 7 Saturda
3 Tuesday

Collection method: (Circle one)

- 1 Recall
- 2 Record

Visit #: ___ ___ ___

To be completed by TNDC:

Date coded: ___ ___ - ___ ___ - ___ ___
m m d d y y

Date Checked: ___ ___ - ___ ___ - ___ ___
m m d d y y

Coder ID#: ___ ___ ___

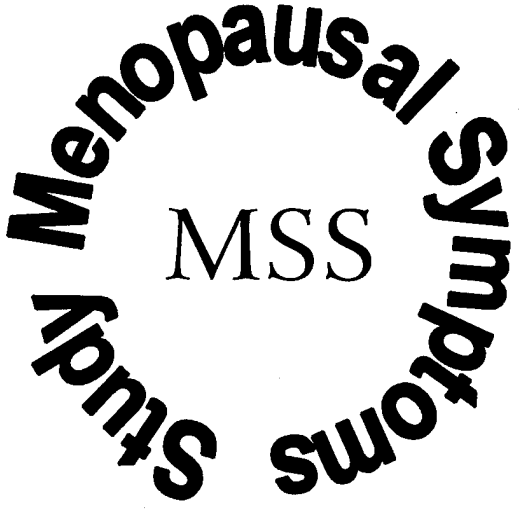
Checker ID#: ___ ___ ___

Form - N-1

F. MANUAL OF OPERATIONS (MOOP)

MENOPAUSAL SYMPTOMS STUDY

MANUAL OF OPERATIONS



Effect of a Soy Dietary on Menopausal Symptoms and Hormones in Women at High Risk of Breast Cancer (MSS)

ROSTER

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2. INTRODUCTION

a. Overview of Manual

This manual describes operations at Tufts University School of Medicine and Memorial Sloan Kettering Hospital for the Menopausal Symptoms Study (MSS) funded by the United States Army under the title "Effect of a Soy Dietary on Menopausal Symptoms and Hormones in Women at High Risk of Breast Cancer". It details those operations that should be performed in a standardized fashion at both sites and includes all study protocols.

b. Data Forms

The following numbered forms will be used to collect participant data.

Form	Description
F1	Screening Questionnaire (probably for Boston only)
F2	Menopausal Symptoms Questionnaire
F3	Medical History Form (completed by ppt in Boston, transferred to form by staff in NYC.
F4	Study Log
F5	Seven Day Daily Symptoms Diary
N1	Food Record/Instructions
N2	Food Questionnaire

Once a patient has been randomized into MSS and assigned a study ID number a folder (or notebook) must be initiated. Paper copies of all numbered forms, laboratory results and study booklets should be filed in the participants folder. MSS forms for all patients screened but not enrolled must also be retained. These do not need to be filed in individual folders, however, should be kept together.

i. Study ID's

Intervention
Intervention Women will be assigned sequential numerical ID's according to the following scheme. Dropouts must be reported to Sloan Kettering in order to maintain the appropriate randomization scheme.

Tufts: ID 's 001-200
NYC: ID's 201-399

Control:
Tufts: ID's 500-599
NYC: ID's 600-799

c. Schedule of Study

i. Time Line

Months

0 to 4	Development and printing of questionnaires Development of data entry forms Development of recruitment materials (letters, flyers, advertisements) Training of study recruiters Write Manual of Operations (MOOP)
5-40	Recruit subjects Begin study Collect and analyze study data serum urine dietary
42-48	Complete laboratory studies Statistical analyses Write final report

3. RECRUITMENT, SCREENING AND ENROLLMENT

a. Overview

All subjects must go through the screening process in order to determine eligibility for their study. The initial screening may take place either over the telephone using the Telephone Screening Questionnaire (F1) or in the clinic. As part of the screening participants must complete the Menopausal Symptoms Questionnaire (F2), Medical History Form (F3) and Seven Day Daily Symptoms Diary (F5).

b. Screening Process

Initial Screen to take place over the Telephone or at the Clinic

1. Introduction/Purpose of Study/Screening Process
2. Complete Screening Questionnaire (F1)
3. If the person is ineligible thank them for their interest in the study. If the person may be eligible ask them if you can send/give them some forms to complete to determine complete eligibility. Explain the forms to be completed and ask the participant to mail them all back after the Seven Day Daily Symptoms Diary is completed.
4. Mail or give to participant
    a. Introductory Letter
    b. Menopausal Symptoms Questionnaire (F2)
    c. Medical History Questionnaire (F3)
    d. Seven Day Daily Symptoms Diary (F5)
    e Food Questionnaire (N2).
    f. Consent Form 1 (Screening)
    g. Self addressed Stamped envelope
5. Review the returned forms (F2, F3, F5) questionnaires for eligibility. (If the participant has not returned the forms within 2 weeks call to see if they have mailed forms or are still interested).
    a. Ineligible: Send a form letter (L1) to the participant thanking them for their interest.
    b. Potential: Determine if participants are eligible for either the control or intervention group. If they are not eligible for either one send them a form letter (L1). If they are eligible for the control group or intervention group call them, describe the study they are eligible for (control or intervention) and schedule a Screening/Baseline Visit (SBV1).

c. General Eligibility Criteria

Subjects: Subjects for this study will be postmenopausal women, ages 48-58 who are at high risk for breast cancer. A total of 200 women will be studied, 100 from Boston and 100 from NYC; from each clinic half of the women (n=50/clinic) will be control subjects who are without menopausal symptoms and half will be women who are experiencing frequent and consistent menopausal symptoms (hot flashes and night sweats). Women will be assigned to the control and intervention groups based on the frequency of hot flashes and night sweats as ascertained by the menopausal questionnaire. Those with ≥5 hot flashes during a 24 hour period will be eligible for the intervention arm of the study. Women with less than one hot flash/night sweat per day will be eligible for the control group.

> *The terms hot flash, hot flush and night sweats are considered to be the same. These are typically sudden episodes of feeling warm, flushing and/or sweating. These events can take place during the day or night.*

Eligibility: Women at high risk for breast cancer as defined by having one or more of the following characteristics: 1) mother or sister with breast cancer, 2) two or more benign breast biopsies, 3) one breast biopsy with diagnosis of proliferative breast disease. Women who have undergone a natural menopause (no periods for at least one year) will be eligible. Women recruited must be within 90-120% of ideal body weight.

d. Exclusion Criteria

Exclusions: Women will be excluded for the following reasons:

1. surgical menopause (no intact ovaries)
2. Use of HRT within the last 6 months.
3. previous breast cancer
4. history of frequent use of antibiotics (>2 times per year).
5. high intake of soy products such as tofu, soybeans, soy flour
6. medications that effect or interfere with hormone metabolism including the following classes of drugs **(expand this list)**
    - Antibiotics
    - Hepatic enzyme inducers i.e. phenobarbital, dilantin, rifampin
    - Hepatic enzyme inhibitors i.e. cimetidine, zantac, tagamet
    - Prostaglandin inhibitors i.e. aspirin
    - Steroids/Hormones i.e. prednisone
    - Antacids
    - Tricyclic antidepressants
    - Blood thinners i.e. coumadin, warfarin

e. Recruitment

i. Tufts

Letters will be sent to all age-eligible, high breast cancer risk patients from the Breast Health Center (BHC) and Dept. of OB/GYN at New England Medical Center Hospital (see sample). Included in the proposal are letters of support from the director of the BHC and the chief of OB/GYN to recruit from their patient populations. A trained recruiter will be available at the clinic site 3 half-days a week (during the scheduled clinic times). Dr. Sajer, will be involved in overseeing the clinic recruitment protocol in the BHC and OB/GYN clinics. Advertisements will be placed in the NEMCH newsletter and local newspapers. Letters will be sent to all OB/GYN physicians in the greater Boston area. Copies of all advertisements/posters must be submitted to the NEMCH HIRC for approval.

ii. Sloan Kettering

Women will be recruited from the Special Surveillance Breast Program and from the Breast Diagnostic and Treatment Center at Memorial Sloan Kettering Cancer Center.

f. Enrollment

Enrollment will take place at the Screening/Baseline Visit 1 (SBV1). A participant is enrolled when she is assigned a study identification number. To ensure that the participant is enrolled corresponding to the preassigned randomization sequence, the next available study identification number must be selected from the Randomization List and the randomization list must be appropriately updated. Verify eligibility for the control or intervention group and have participant sign the appropriate consent forms.

g. Randomization

A randomization list will be developed by the biostatistical office at Memorial hospital. Random assignment will be made using a computer algorithm to ensure a balanced assignment for every 10 patients entered, and will take into account patient dropout. Dietary supplement bars will be labelled and pre-packaged at Tufts University and these efforts will be supervised by Lisa Gualtieri, Laboratory Manager. All study personnel will be blinded as to assignment of soy or placebo bars.

4. STUDY PROTOCOL

a. Overview

The purpose of this seven month study is to determine the effect of a soy dietary supplement bar on menopausal symptoms in women at increased risk for breast cancer who are experiencing frequent menopausal symptoms.. Women will be randomized using a double-blind, cross-over design to receive either a placebo bar or a soy dietary supplement bar for three months with a one month washout between protocols. A matched group of control women at high risk for breast cancer but with low symptomatology will be studied.

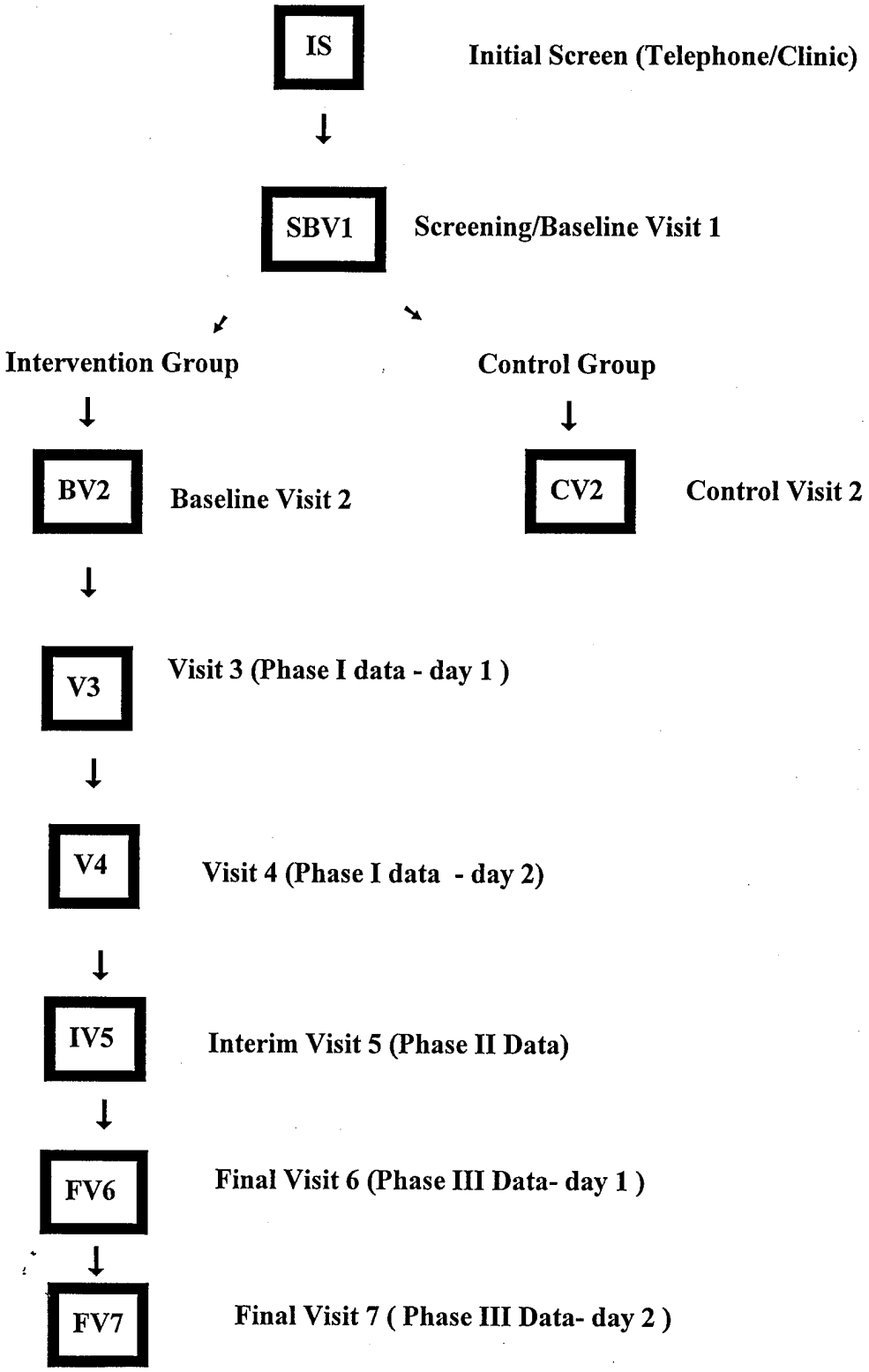
Intervention Group: Baseline blood and urine collections will be done on all women. At the two baseline visits SBV1 and BV2 blood samples will be taken (40 ml/SBV1, 30 ml/BV2) for measurement of routine blood chemistries and hormone levels and a first morning urine sample will be collected on the day of BV2. During Phase I, women are randomally assigned to either the soy supplement group or placebo group for three months. Subjects consume two soy or placebo bars each day. At the end of Phase I blood samples are taken on two days approximately one week apart (V3 and V4) and a first morning urine sample is collected on the morning of V4. During the fourth month of the study Phase II (Washout) women do not consume any supplement bars. For the remaining three months of the study (Phase III) women who were given the soy bar are switched to the placebo bar and women who were given the placebo are switched to the soy bar. At the end of the study blood and urine data are be collected as before at visits FV6 and FV7. The length of the dietary intervention study is 7 months.

Dietary data is collected at baseline, and at the end of Phases I, II and III. At baseline a food frequency questionnaire (N2) will be completed; this takes approximately 20 minutes. A three day food record (N1)will be kept at baseline, and at the end of Phases I, II and III. This requires that a subject write down all food and beverages consumed during a three day period and takes approximately 10-20 minutes each day. Subjects will receive instructions on how to keep a food record, and will review each completed food record with the as certified dietary data collector.

Throughout the study subjects record information about their menopausal symptoms. A menopausal symptoms questionnaire (F2) is completed at baseline and at the end of each phase. Women keep a daily study log to record the number of hot flashes they experience each day, and to track dietary supplement bar consumption. Also, at baseline and at the end of each phase, women keep a detailed seven day diary indicating the time and intensity of each hot flash experienced during a specified seven day period.

Control Group: Blood samples are taken on two days approximately 1 week apart SBV1 and CV2 (40 ml/SBV1 and 30 ml/CV2) for measurement of routine blood chemistries and hormones, and first morning urine samples will be collected on the day of CV2 for measurement of phytoestrogens. Women keep a daily diary of symptoms (hot flashes/night sweats) during this time. Dietary intake data is collected using a food frequency questionnaire and a three day food record.

Study Overview-Flow Chart



c. Distribution of Soy Dietary Supplements

Dietary supplement bars are packaged in one 4 week supplies (56 bars) and distributed to participants monthly at regularly scheduled study visits and mailed to participants at home between visits.

d. Collection of Study Data

i. Blood Samples

Intervention Group: Blood samples are required at SBV1, BV2, V3, V4, FV6 and FV7.
Control Group: Blood samples are required at SBV1 and CV2,

ii. Urine Specimens

Intervention Group: At BV2, V4 and FV7 subjects will provide a specimen from the first morning void .
Control Group: At CV2 subjects will provide a specimen from the first morning void .

If the participant forgets to collect the first morning void a specimen is collected at the time of the visit.

ii. Food Records (N1)

Intervention Group: Three day food records are kept at baseline, and at the end of Phases I, II and III.

Control Group: One three day food record is kept at baseline.

At the Screening/Baseline Visit **SBV1** participants are given specific instructions on how to keep a three day food record by study personnel who have been trained and certified in dietary assessment for this study. Participants are provided with a scale to assist them in recording accurate amounts of food consumed.

iii. Seven Day Daily Symptoms Diary (F5)

This booklet is used to record the time and intensity of all hot flashes experienced during a specified period. The seven day daily symptoms diary is kept by all participants as part of the screening process to determine eligibility for the control or intervention group.

Control Group: The seven day daily symptoms diary is kept during the first week of the study.
Intervention Group: The seven day daily symptoms diary is kept at baseline and at the end of Phases I, II and III.

iv. Study Log (F4)

The Study Log is used only by the women in the intervention group. Each day throughout the study women record the number of hot flashes experienced during waking and sleeping hours and indicate the number of soy bars eaten. Subjects are given a new study log each for each month of the study and at the end of each month should mail the completed study log to the study coordinator at each clinic.

5. LABORATORY PROCEDURES

a. Collection of Blood

Blood samples are required at SBV1, V2, V3, V4, V6 and FV7.

i. Collection Tubes/Supplies

Blood will be collected in 10 ml SST red top vacutainers . Serum will be stored in 17x100mm polystyrene tubes and 17 x100 mm tubes 1/out caps will be needed for processing.

Supplies can be ordered from local scientific supply companies. The items needed are desribed in the Fisher Scientific catalog as described below:

Item	Description	Catalog#	Price
SST Serum Separation Tube Red stopper lubricated w/silicone	B-D No. 367815 13x100	02-683-94	Case of 1000/ $252.40
Sterile Plastic tubes with snap caps	17x100	14-956-6A	Case of 1000/ $135.50
Sterile Plastic tubes w/out caps	17x100	14-956-6C	Case of 1000/ $90.00

ii. Procedures for Obtaining Specimens

All blood samples will be obtained by venipuncture. At SBV1 one 10 ml red top tube (SST) will be taken and sent to clinical chemistry for routine blood chemistry analyses blood and three 10 ml red top SST (serum separator tubes) will be taken for hormone analyses. At all subsequent visits three 10 ml red top SST (serum separator tubes) will be taken for hormone analyses.

Boston: Blood samples will be taken by licensed phlebotemists at the Blood Drawing Laboratory located in the South building. Subjects will either transport the blood to our laboratory or study personnel will accompany the participant to Blood Drawing and then take the sample to our Laboratory for processing and storage . Study personnel will take Universal Precautions when handling and/or transporting blood samples.

New York City: Blood samples will be taken by licensed phlebotemists or trained personnel in the outpatient area and then processed in the laboratory. Study personnel will take Universal Precautions when handling and/or transporting blood samples.

iii. Processing of Blood

Serum specimens should be processed as follows:

1. Allow tubes to sit for 20-30 minutes (but no longer than 2 hours) in a test tube rack.
2. With the cap in place, centrifuge at 2500 RPM for 5 minutes. (Tufts use the Beckman tabletop centrifuge set at speed 6.8 brake set on high. Centrifuge must be balanced prior to spinning.
3. Pour serum from all three tubes into one plastic 17x100 mm tube, Serum is aliquoted by pipette as follows:
   a.. 5.0 ml is put into a 17 x 100 mm tube for hormone analyses by Dr. Longcope.
   b. 5.0 ml is put into a 17 x 100 mm tube for storage at Tufts.
   c. Remaining serum is measured and put into a 17 x 100 mm tube for storage.

(1) Labelling of Samples

Labels will be preprinted for each subject. *Check to be sure that the subject ID and visit number on the label correspond to the patient who had blood drawn.* It is necessary for the clinic to record the date on each label. Also, the ml of remaining serum must be written in on Tube #3. After labels are placed on polystyrene tubes they must be secured by clear "non-temperature sensitive" tape to the tube. This is extremely important because the laser printer labels will not adhere properly to the tubes in the freezer.

If the preprinted labels are lost or damaged the following information must be written clearly on the tube, following the same format as the preprinted labels.

Study Name	=	MSS in the upper left hand corner,
Site	=	BOS (Boston), NYC (New York City) next to
Sample ID	=	Subject ID ### - Visit - Sample Number
		Subject ID: Boston 001-200, NYC 201-399
		Visit: SBV1,BV2,V3,V4, FV6,FV7
		Sample #: 1,2,3,4,5,6
Date of sample	=	MM/DD/YY in the lower left corner
Serum Identifier	=	Serum Hormones or Serum Storage in the upper right hand corner
Tube Number	=	1,2,3
Amount of serum	=	amount in ml in lower right hand corner

EXAMPLE:

A set of labels for subject 1 from Boston taken at visit SBV1 on 1/1/95 would look like this:

MSS-BOS	Serum Hormones
001-SBV1-1	1
Date 1/1/95	5.0 ml

MSS-BOS	Serum Storage
001-SBV1-1	2
Date 1/1/95	5.0 ml

MSS-BOS	Serum Storage
001-SBV1-1	3
Date 1/1/95	___ ml

(2) Laboratory Sample Log Books/Storage Boxes

Each tube is logged into the laboratory Sample Log Book. For each sample the following information is entered in the LOG, Study ID, Visit, Sample #, Date, Tube #, ml of blood, and Storage Box # and comments indicating any problems or anything unusual about the sample.

Study logs will be entered into PARADOX at each clinic on a weekly basis.

EXAMPLE:

MSS STUDY- BOSTON
SERUM LOG

ID	Visit	Sample#	Date	Tube#	Amount	Box	Comments
001	SBV1	1	1/1/95	1	5.0	H01	serum clot
001	SBV1	1	1/1/95	2	5.0	S01	serum clot
001	SBV1	1	1/1/95	3	7.5	S01	serum clot

At each clinic there will be two sets of storage boxes- those for tubes labelled Serum Hormones and those for tubes labelled Serum Storage. The Storage boxes are labelled on the outside with the following information, Study Name, Clinic, Box Title (Serum Hormones/Serum Storage) and Box #. Box #'s should be in consecutive, numerical order, for example, H01, H02,... S01, S02...

MSS-BOS	Hormones
Serum	Box H01

MSS-BOS	Storage
Serum	Box S01

Storage boxes and dividers for storing the serum tubes can be ordered from Fisher Scientific:

Item	Revco #	Catalog No.	Price	Number/Clinic
Box	5956	11-678-24B	Package of 12/$28.00	~2 packages
Dividers	5959	11-678-26D	Package of 12/$15.00	~2 packages

iv. Problems with Handling Blood Specimens

Reliable tests can only be obtained from properly collected specimens. Some common problems are:

1. Hemolysis; Blood which is hemolyzed during collection may interfere with some analyses. Hemolysis my be caused by overcentrifugation, excessive shaking of the sample, freezing cells and forcing blood through the venipuncture needle.

2. Hemoconcentration: If the tourniquet is left on too long during venipuncture, stasis may occur in the vein and blood constituents may be more concentrated than normal.

3. Overcentrifugation: Blood specimens should be centrifuged only after standing for 20-30 minutes to allow clotting to occur. Centrifugation should be gentle to avoid cell damage and serum should be separated as soon as possible from the cells. A serum separator in the tube physically separates the serum from red blood cells.

4. Evaporation: Blood and urine samples should remain capped until the time of testing to prevent evaporation. Evaporation can alter pH or HCO_3 and cause concentration.

5. Contamination: To avoid contamination or dilution, clean dry pipettes should be used to aliquot each specimen.

6. Thawing: Freezers should be monitored daily for temperature drift since large temperature swings affect samples adversely.

v. Storage of Blood

Serum tubes are stored in storage boxes at -70 °C and should be frozen immediately.

vi. Shipment of Blood

Blood should be shipped to Tufts approximately every 3 months.

vii. Shipping Logs

A separate shipping log must be included for each box of serum.

viii. Packing the Specimens

Specimens should be packed in enough dry ice to last 24 hours. The shipping logs should be placed in a plastic zip loc bag inside the box or in an envelope on the outside of the box.

ix. Shipping Procedures

Bloods should be shipped using an overnight delivery service that guarantees delivery before noon. Samples should only be shipped Monday-Wednesday. Prior to shipping it is imperative that a representative from Tufts be contacted (Ann LaBrode, Christina Sadlow, Margo Woods or Lisa Gualtieri) to be sure that someone will be there to check on arrival of samples.

x. Analysis of Samples (Tufts/NYC)

All hormones determinations will be done in the laboratory of Dr. Chistopher Longcope at the University of Massachusetts Medical School. As described blood samples are collected on two days (approximately one week apart) at baseline and at the end of Phases I and III. Pooled serum samples from the two days are used for hormone analyses. Serum hormone measurements are carried out for estrone, estradiol, free estradiol, estrone sulfate, androstenedione and testosterone by radioimmunoassay involving solvent extraction and celite chromatography. All samples are run in duplicate. The FSH determinations are performed by a double-antibody radioimmunoassay utilizing a kit obtained from Radioassay Systems Laboratories Inc., Carson CA.

xi. Quality Control Procedures

Blinded duplicate quality control samples will be sent to Dr. Longcope's laboratory for analysis and a quality control report will be written.

a. Collection of Urine

i. Collection Bottles/Supplies

Urine will be collected in 4 oz/118 ml specimen collection containers and stored in 30 ml polyethylene sample bottles. Supplies can be ordered from local scientific supply companies and are described in the Fisher Scientific catalog as indicated below:

Item	Description	Catalog Number	Price	Number/Clinic
4 oz./118ml Specimen container	Non-sterile/ white cap	14-375-148	Case of 500/ $96.50	~ 1 case
Nalgene* Transculent high density polyethylene storage bottles	1 oz/30 ml cap 28mm	03-313-4A Nalge 2189-0001	Case of 72/ $64.20	~600/9 cases

ii. Procedures for Obtaining Specimens

At SBV1, V3 and FV6 each participant should be given an instruction sheet and a small container for the collection of a first morning voided urine specimen on the mornings of BV2, V4 and FV7. It is not necessary to collect the entire first morning void, only a sufficient volume (e.g., 100 ml) for the required urine aliquots. The sample should be collected before the participant eats or drinks anything. The container should have a label marked with the Study ID, Visit number for urine collection, urine collection number (1,2,3) and date of urine collection. In addition the time of the collection should be recorded on the label. The specimen should be kept refrigerated or cold until it is turned in at the clinic.

Important: Prior to the urine collection 0.1 grams of ascorbic acid powder must be added to each urine collection container. This prevents the breakdown of the urinary phytoestrogens.

iii. Processing of Urine

Specimens should be stored in the refrigerator until they can be processed. Specimens must be processed within 24 hours. Processing should follow these steps:

1. Mix urine well to suspend sediment.

2. Aliquot 30 ml of urine into each of the three urine storage bottles.

3. Add 0.3 ml of 10% Sodium Azide solution to each 30 ml aliquot. (10% Sodium Azide solution is prepared by mixing 10 g of Sodium Azide w/100 ml water).

4. Samples are stored at -70° until shipment to Tufts.

iv. Labelling of Samples

Labels will be preprinted for each subject. *Check to be sure that the subject ID and visit number on the label correspond to the patient who brought in the urine sample.* It is necessary for the clinic to record the date on each label. After labels are placed on the storage bottles they must be secured by clear "non-temperature sensitive" tape to the tube. This is extremely important because the laser printer labels will not adhere properly to the tubes in the freezer.

If the preprinted labels are lost or damaged the following information must be written clearly on the storage bottles, following the same format as the preprinted labels.

Study Name	=	MSS in the upper left hand corner,
Site	=	BOS (Boston), NYC (New York City) next to
Sample ID	=	Subject ID ### - Visit - Sample Number
		Subject ID: Boston 001-200, NYC 201-399
		Visit: BV2,V4, FV7
		Sample #: 1,2,3
Date of sample	=	MM/DD/YY in the lower left corner
Urine Identifier	=	the word "Urine" in the upper right hand corner
Bottle Number	=	1,2,3
Amount of urine	=	amount in ml (30) in lower right hand corner

EXAMPLE:

A set of urine labels for subject 1 from Boston for a urine collection done on BV2 on 1/7/95 would look like this:

MSS-BOS	Urine/Phytoes
001-BV2-1	1
Date 1/7/95	30 ml

MSS-BOS	Urine Creat
001-BV2-1	2
Date 1/7/95	30 ml

MSS-BOS	Urine Storage
001-BV2-1	3
Date 1/7/95	30 ml

(2) Laboratory Sample Log Books/Storage Boxes

Each Bottle is logged into the laboratory Sample Log Book. For each sample the following information is entered in the LOG, Study ID, Visit, Sample #, Date, Bottle #, ml of urine, and Storage Box # and comments indicating any problems or anything unusual about the sample. Study logs will be entered into PARADOX at each clinic on a weekly basis.

EXAMPLE:

MSS STUDY- BOSTON
URINE LOG

ID	Visit	Sample#	Date	Bottle#	Amount	Box	Comments
001	SBV1	1	1/1/95	1	5.0	P01	
001	SBV1	1	1/1/95	2	5.0	C01	
001	SBV1	1	1/1/95	3	5.0	S01	

At each clinic there will be three sets of storage boxes- those for bottles labelled "Urine Phytoes", those for bottles labelled "Urine Creatinine" and those for bottles labelled "Urine Storage". The Storage boxes are labelled on the outside with the following information, Study Name, Clinic, Box Title (Serum Hormones/Serum Storage) and Box #. Box #'s are in consecutive, numerical order, and the box number is preceded by a P for Phytoes, C for Creatinine or S for Storage, for example:

MSS-BOS	Phytoes
Urine	Box P01

MSS-BOS	Creatinine
Urine	Box C01

MSS-BOS	Storage
Urine	Box S01

iv. Storage of Urine

Urine is stored in the freezer at -70° .

vi. Shipment of Urine

Urine should be shipped to Tufts approximately ever 3 months.

vii. Shipping Logs

A separate shipping log must be included for each box of urine

viii. Packing the Specimens

Specimens should be packed in enough dry ice to last 24 hours. The shipping logs should be placed in a plastic zip loc bag inside the box.

ix. Shipping Procedures

Urine should be shipped using an overnight delivery service that guarantees delivery before noon. Samples should only be shipped Monday-Wednesday. Prior to shipping it is imperative that a representative from Tufts be contacted (Ann LaBrode, Christina Sadlow, Margo Woods or Lisa Gualtieri) to be sure that someone will be there to check on arrival of samples.

x. Analysis of Samples (Tufts)

Phytoestrogens will be analyzed in the laboratory of Dr. Herman Adlercreutz with whom our group has collaborated for over 15 years. The phytoestrogens to be measured are matairesinol, secoisalariciresinal, enterolactone, enterodiol, genestein, daidzein, equol and o-desmethylangolensin). Urine samples will be collected from intervention women at baseline and at the end of Phase I and Phase III. While urine samples will be collected on all intervention women, at this time only 25% of the population will be analyzed due

to the high cost of the analyses. The remaining samples will be stored and additional funding will be sought.

The determination of the urinary lignans and phytoestrogens will allow us to: 1) validate baseline intake of these compounds. 2) verify compliance with the dietary supplement protocol and 3) correlate symptoms with urinary excretion of these individual compounds. From each urine sample 0.33% of the sample is used for analysis. The urine is buffered by adding 1.5M acetate buffer pH3.0. The volume of buffer added is 10% of the urine volume. ^{3}H-estrone glucuronide (10,000 DPM) is added as an internal standard to correct for losses during the analysis procedure.

Sep-Pak C^{18} extraction: Before use, the Sep-Pak column is washed with 5 ml of methanol and 10ml water. The urine sample is added and the column is washed with 5 ml of 0.15 M acetate buffer pH 3.0 and the column is eluted with 3 ml of methanol. To the methanol eluate a total of 1.2 ml of water is added. The 4.2 ml sample is added to a DEAE-Ac- column which has been prepared in a pasteur pipette in 70% methanol. The column is eluted by adding 4 ml of 70% methanol. This fraction is discarded. The final elution is performed with 10 ml of 0.3 M LiCL in 70% methanol. This fraction contains the conjugates of the lignans and phytoestrogens. Because the amount of free lignans and isoflavones is negligible only the final elute is used for further analysis. The deuterated internal standards enterolactone, enterodiol, matairesinol, equol, daidzein O-desmethylangolensin and genistein are added. The methanol is then evaporated off leaving only the water. To this specimen additional water is added to make a total volume of 10 ml and then 1 ml of 1.5 M acetate buffer, pH 3.0 is added. The sample is transferred to a Sep-Pak column that has been previously washed with 5 ml of 0.15 M acetate buffer pH 3.0. The sample is then eluted with 3 ml of methanol and the eluate is evaporated to dryness.

Hydrolysis of the conjugates: 5 ml of 0.15 M acetate buffer pH 4.1 containing 25 mg ascorbic acid and 500 ul of Helix Pomatra (Sigma Chemical Co., St. Louis) enzyme extract is added to the evaporate. The sample is incubated for 12 hours at 37°C.

Separation of free lignans and phytoestrogens: The sample is added to washed (5ml water) Sep-Pak column and eluted with 3 ml of methanol. The eluate is added to a QAE-A column. The column is first eluted with 4 ml of methanol. This fraction #1 contains enterolactone, enterodiol, matairesinol, equol and estrogens. The second fraction (fraction #2) is eluted from QAE-Ac column by adding 7 ml of 0.2 M acetic acid in methanol (1:85 v/v). Fraction #2 contains o-desmethylangolensin (DAM), daidzein (DA) and genistein (ge). Fraction #1 is evaporated to dryness and 4 ul ml methanol and 0.1 ml water are added to the residue. The sample (0.5ml) is added to a QAE-Ac column (4 x 0.5 cm). The column is initially eluted with 4 ml of 80% methanol and this fraction containing the estrogens is discarded. The column is then eluted with 5 ml 0.1 M acetic acid in methanol: water (4.1). This fraction contains enterolactone, enterodial, equol and matairesinol.

Analysis for lignans and phytoestrogens: Fractions #1 containing lignans plus equol (isoflavone) or fraction #2 containing isoflavones are separated by evaporating the fractions to dryness and adding 100 ul of pyridine/HMDS/TMS (9:3:1) and incubating for 30 minutes at room temperature. The fractions are then evaporated to dryness and residue is dissolved in 100-300 ul of hexane.

The fractions in hexane are then analyzed by GC/MS as previously described (45). The identification of each compound is based upon its gas chromatographic retention time on two capillary columns of different polarity, the complete mass spectra and selection ion monitoring responses relative to the authentic standards. The quantification is achieved by relating the peak areas of the specimen to the peak area of known amounts of the internal standards.

xi. Quality Control Procedures

Blinded duplicate samples will be sent and analyzed for a measure of quality control.

c. Universal Precautions for Handling Biological Specimens

Biological specimens may contain infectious agents which may be harmful to specimen handlers. The CDC strongly recommends universal blood and body fluid precautions when handling all patient specimens. These include:

1. Use of protective barriers to prevent exposure of skin or mucous membranes, including gloves, goggles or safety glasses. These should be disposed of properly after use.

2. Hands should be washed immediately after removing gloves, and any contaminated skin surface should be washed immediately and thoroughly.

3. Use precautions to avoid injuries from sharp, contaminated instruments such as needles.

4. Do not contaminate the outside of specimen containers, labels or forms.

5. Never pipet by mouth. Only pipets with safety bulbs or automatics pipets should be used.

6. Biological specimens and contaminated articles including blood tubes and pipets must be placed in a special biohazard bag for disposal.

7. The lab work area should be disinfected before and after use and whenever a spill occurs. An appropriate solution is a 5-10% solution of sodium hypochlorite or a good commercial lab disinfectant.

3. Soy Bars

The Soy and Placebo bars are produced by Protein Technologies and have the following nutrient composition.

	Soy Bar	Placebo Bar
Serving (g)	65.0	56
Moisture (g)	6.5	6.7
Fat (g)	1.3	0.9
Protein (g)	15.1	16.4
Ash (g)	2.0	2.1
Carbohydrate (g)	31.1	29.9
Calories (kcal)	196.	194
Calcium (mg)	711.	497
Iron (mg)	4.0	2.1
Phosphorous (mg)	502	304
Sodium (mg)	168	106
Folic Acid (mcg)	142	158
Pantothenic Acid (mg)	.94	.78
Vitamin B6 (mg)	.26	.23
Riboflavin (mg)	.45	.35
Thiamin (mg)	.23	.14
Vitamin A (IU)	668	809
Vitamin B12 (mcg)	1.29	3.19
Vitamin C (mg)	1.41	1.67
Vitamin D (IU)	153	164

G. CONSENT FORM - TUFTS

Tufts University School of Medicine
New England Medical Center Hospital

CONSENT FORM 1 (Screening)
Menopausal Symptoms Study
Principal Investigator: Margo N. Woods, D.Sc.
Medical Monitor: Sherwood L. Gorbach, M.D.

Purpose: The purpose of this research study is to investigate the effect of dietary soy on hormone levels and menopausal symptoms in women at increased risk for breast cancer. This study is funded by the United States Army Medical Research, Development, Acquisition and Logistics (USAMRDL) Command (Provisional). This Study will be conducted at the Breast Health Center located in the South Building at New England Medical Center, 750 Washington Street. Boston, MA 02111.

Rationale: Menopausal symptoms have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms there are concerns about the affect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

Eligibility/Study Requirements: To determine eligibility for this study you will be asked to complete a medical history questionnaire, a menopausal symptoms questionnaire, a one week daily diary of hotflashes/night sweats and a food frequency questionnaire. If you are eligible and agree to participate in this study a screening/baseline blood sample will be taken (40 ml -approximately 4 tablespoons) and analyzed for routine blood chemistries and hormone levels. This screening visit will take approximately 2 hours. Based on the results of the menopausal symptoms questionnaire and the daily diary of hot flashes you will be eligible for either the control group or the intervention group. If you are assigned to the control group you will be asked to participate in a one week study and baseline data will be collected as described in Consent Form 2. If you are assigned to the intervention group you will be asked to participate in a 7 month dietary intervention study as described in Consent Form 3.

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 1 (Screening)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

Blood Drawing: The total amount of blood to be taken for the screening is 40 ml. (approximately 3 tablespoons).

Risks: The only risks associated with this part of the study are those associated with blood drawing, which in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing when performed by someone who has been specially trained and has experience in drawing blood decreases these risks.

Benefits: The data obtained in this study may be of no direct benefit to the participant but may yield insight into the effects of diet on hormone metabolism and its possible role in the cause and development of breast cancer.

Whom to Contact: If you have any questions about the study or experience any problems or injury or illness during it, you should call one of the following persons:

	Days	Evenings
Dr. Margo Woods	617-636-0809	617-646-6059
Dr. Sherwood Gorbach	617-636-5811	617-636-5111, beeper #1193

Stipend: Subjects will not be paid for participating in this initial screening. Stipends will be paid to Control and Intervention subjects as explained in Consent Forms 2 and 3. There will be no additional costs to the participant for participating in this study.

It is the policy of the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC) that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 1 (Screening)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

PARTICIPANT'S STATEMENT

I have read this consent form and have discussed with Dr._____ or his representative the procedures described above. I have been given the opportunity to ask questions, which have been answered to my satisfaction. I understand that any questions I might have asked will be answered verbally or, If I prefer, with a written statement understand that I will be informed of any new findings developed during the course of this research study.

I understand that my participation in this study is voluntary. I understand that I may refuse to participate in this study. I also understand that if, for any reason, I wish to discontinue my participation in this study at any time, I will be free to do so, and this will have no effect on my future care or treatment by my physicians or this hospital.

I understand that I am authorized all necessary medical care for injury or illness which is the proximate result of my participation in this research. Other than medical care that may be provided (and the stipend specifically stated in this consent form) there is no compensation available for my participation in this research study, however, I understand that this is not a waiver or release of my legal rights.

If I have any questions concerning my rights as a research subject in this study, I may contact the Human Investigation Review Committee at 617-636-7512. This office is located at 35 Kneeland Street, 6th floor Boston, MA 02111.

I have been fully informed of the above-described study with its risks and benefits, and I hereby consent to the procedures set forth above. I have received a copy of this consent form.

I understand that as a participant in this study my identity and my medical records and data relating to this research study will be kept confidential, except as required by law, and except for inspections by the study sponsor, the United States Army Medical Research, Development, Acquisition and Logistics (USAMRDL) Command (Provisional).

_____ _____

Participant Signature Date

_____

Participant name (printed) Address (street or PO Box, city, zip code)

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 1 (Screening)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

I have fully explained to _____ the nature and purpose of this above described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____
Principal Investigator/Representative

_____
Witness Signature

_____
Witness Printed name

_____
Date

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Tufts University School of Medicine
New England Medical Center Hospital

CONSENT FORM 2 (Control)
Menopausal Symptoms Study
Principal Investigator: Margo N. Woods, D.Sc.
Medical Monitor: Sherwood L. Gorbach, M.D.

Purpose: The purpose of this research study is to investigate the effect of dietary soy on hormone levels and menopausal symptoms in women at increased risk for breast cancer. This study is funded by the United States Army Medical Research, Development Acquisition and Logistics (USAMRDAL) Command (Provisional). This Study will be conducted at the Breast Health Center located in the South Building at New England Medical Center, 750 Washington Street, Boston, MA 02111.

Rationale: Menopausal symptoms such as hot flashes and night sweats have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms there are concerns about the affect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

Eligibility: Based on the Menopausal Symptom Questionnaire you are eligible to participate in the Control Group for this study. This is because you are either not experiencing any hot flashes or you are not having hot flashes every day. The term "Control Group" is used because the purpose of the group is to collect data (blood hormones and urinary phytoestrogens) in order establish baseline data for this values in postmenopausal women with no menopausal symptoms or low levels of menopausal symptoms and to compare these values to women who are experiencing frequent menopausal symptoms.

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 2 (Control)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

Study Requirements: If you choose to participate, the length of this study is approximately one week. During this week you will keep a three day food record and complete a one week daily diary of the number of hot flashes you have. Approximately one week after the screening visit a blood sample (30 ml) will be taken for measurement of hormones. On this morning you will be asked to collect all urine from your first morning void. You will be provided with a bottle for the collection, a cooler and ice pack. The urine must be kept cold until you come to the clinic.

Blood Drawing: The total amount of blood taken for this study will be 30 ml (approximately two tablespoons).

Risks: Blood drawing, in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing performed by someone who is specially trained and experienced in blood drawing decreases these risks.

Benefits: The data obtained in this study may be of no direct benefit to the participant but may yield insight into the effects of diet on hormone metabolism and its possible role in the cause and development of breast cancer. Data from the blood analyses will be available to you.

Whom to Contact: If you have any questions about the study or experience any problems or injury or illness during it, you should call one of the following persons:

	Days	Evenings
Dr. Margo Woods	617-636-0809	617-646-6059
Dr. Sherwood L Gorbach	617-636-5811	617-636-5111 beeper #1193

Stipend: You will receive $20 upon completion of the study. If you withdraw early from the study you will not receive any payment. There will be no additional costs to the participant for participating in this study.

It is the policy of the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC) that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

_____ _____

Participant's Initial Date

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 2 (Control)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

PARTICIPANT'S STATEMENT

I have read this consent form and have discussed with Dr._____ or his representative the procedures described above. I have been given the opportunity to ask questions, which have been answered to my satisfaction. I understand that any questions I might have asked will be answered verbally or, If I prefer, with a written statement. I understand that I will be informed of any new findings developed during the course of this research study.

I understand that my participation in this study is voluntary. I understand that I may refuse to participate in this study. I also understand that if, for any reason, I wish to discontinue my participation in this study at any time, I will be free to do so, and this will have no effect on my future care or treatment by my physicians or this hospital.

I understand that I am authorized all necessary medical care for injury or illness which is the proximate result of my participation in this research. Other than medical care that may be provided (and the stipend specifically stated in this consent form) there is no compensation available for my participation in this research study, however, I understand that this is not a waiver or release of my legal rights.

If I have any questions concerning my rights as a research subject in this study, I may contact the Human Investigation Review Committee at 617-636-7512. This office is located at 35 Kneeland Street, 6th floor Boston, MA 02111.

I have been fully informed of the above-described study with its risks and benefits, and I hereby consent to the procedures set forth above. I have received a copy of this consent form.

I understand that as a participant in this study my identity and my medical records and data relating to this research study will be kept confidential, except as required by law, and except for inspections by the study sponsor, the United States Army Medical Research, Development Acquisition and Logistics (USAMRDAL) Command (Provisional).

_____ _____

Participant Signature - Date

_____

Participant name (printed) Address (street or PO Box, city, zip code)

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Consent Form 2 (Control)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

I have fully explained to _____ the nature and purpose of this above described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____
Principal Investigator/Representative

_____
Witness Signature

_____
Witness Printed name

_____
Date

APPROVED: 12/13/94
VALID THROUGH: 12/13/95

Tufts University School of Medicine
New England Medical Center Hospital

CONSENT FORM 3 (Intervention Group)
Menopausal Symptoms Study
Principal Investigator: Margo N. Woods, D.Sc.
Medical Monitor: Dr. Sherwood I. Gorbach, M.D.

Purpose: The purpose of this study is to investigate the effect of dietary soy on hormone levels and menopausal symptoms in women at increased risk for breast cancer. This study is funded by the United States Army Medical Research, Development, Acquisition and Logistics (USAMRDAL) Command (Provisional). This Study will be conducted at the Breast Health Center located in the South Building at New England Medical Center, 750 Washington Street, Boston, MA 02111.

Rationale: Menopausal symptoms such as hot flashes and night sweats have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis and menopausal symptoms there are concerns about the affect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

This 7 month dietary intervention study will use a random cross- over design to determine the effect of a soy dietary supplement on menopausal symptoms and endogenous hormones. The term "intervention" or "intervention group" means that something is being changed in order to see what affect it might have; in this study subjects will be asked to change their eating habits by eating a dietary soy supplement bar, in order to see whether the dietary soy supplement has any affect on hormones or menopausal symptoms. In Phase I of this study one group of subjects will be given the soy dietary supplement bar for the first three months of the study and the other group of subjects will be given a bar that is very similar to the dietary soy bar, except that it does not contain soy. (This bar is called the placebo bar because it is not anticipated that this bar will have any affect on hormone levels or menopausal symptoms.) The placebo bar will be identical in taste and appearance to the soy bar). For the next four weeks of the study (Phase II)

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participants will not consume any supplement bars. This period is called a washout. For the last three months of the study (Phase III) the group that received the soy bar in the first part of the study will be given the placebo bar and the group that received the placebo bar for the first part of the study will be given the soy bar. This type of study is called a cross-over study. Half of the subjects will begin by eating the soy bar first and half will begin by eating the placebo, and the way that subjects are assigned to each group is by chance. Subjects will not be told which bar they were receiving until the entire study has been completed by all subjects.

Eligibility: Based on the Menopausal Symptom Questionnaire and the Daily Symptoms Diary you are eligible to participate in the Intervention Group for this study. This is because you are experiencing more than five hot flashes each day.

Study Requirements: The length of this study is 7 months. During the first week of the study you will be asked to keep a three day food record. This requires that you write down all food and beverages consumed during a specified three day period and takes approximately 10-20 minutes each day. You will receive instructions on how to keep a food record, and will review the completed food record with the study coordinator. Also, during this week you will be asked to complete the daily symptoms diary. This will take a few minutes each day. Within one week of your screening/baseline blood sample another baseline blood sample (30ml or two Tablespoons) will be taken for measurement of hormones. On this morning you will also collect all of the urine from your first morning void. You will be provided with a bottle for the urine collection, a cooler and ice pack. The sample must be kept cold until you come to the clinic. At this visit you will be randomly assigned to either the soy supplement group or placebo.

Phase I: During Phase I you will consume two soy or placebo bars each day for three months. You will not be told which bar you are given until all the entire study has been completed by all participants. The study investigators and study coordinator will not know whether participants are consuming the soy or the placebo bar until the entire study has been completed by all participants. Each day you will be asked to record the number of hot flashes you have and indicate if both soy bars were eaten. You will record this information in the Study Log booklet. At the end of Phase I (Weeks 11-13) two blood samples (30 ml or two tablespoons/sample) will be taken on two days (within one week) and a urine collection will be done as before. During the last week of Phase I you will be asked to complete a Three Day Food Record, the Daily Symptoms Diary, and the Menopausal Symptoms Questionnaire. Finally, at the end of Phase I you will be asked to return any supplement bars that you did not eat.

Phase II: During this 4 week washout period you will not be consuming any supplement bars. You will be asked to keep track of the number of hot flashes using the Study Log and during the last week of Phase II you will be asked to complete a Three Day Food Record, the Daily

Symptoms Diary and the Menopausal Symptoms Questionnaire.

Phase III: At the beginning of Phase III you will return to the clinic to review the food record with the study coordinator and to pick up your supplement bars for Phase III. If you were given the Soy Bar during Phase I you will consume the Placebo bar during Phase III, and if you were given the Placebo Bar during Phase III you will consume the Soy Bar during Phase II. You will not be told which bar you are given until all the entire study has been completed by all participants. The study investigators and study coordinator will not know whether participants are consuming the soy or the placebo bar until the entire study has been completed by all participants. You will consume two soy or placebo bars each day. Also, each day you will be asked to record the number of hot flashes you have and indicate if both soy bars were eaten. You will record this information in the Study Log booklet. At the end of Phase III (Weeks 11-13) two blood samples (30 ml or two Tablespoons/sample) will be taken on two days (approximately one week apart) and a urine collection will be done as before. Also during the last week of Phase III you will be asked to complete a Three Day Food Record, the Daily Symptoms Diary, and the Menopausal Symptoms Questionnaire. Finally, at the end of Phase III you will be asked to return any supplement bars that you did not eat.

Dietary Supplement: The soy and placebo bars to be used in this study were developed by Protein Technologies International, St. Louis, MO. The soy supplement bar and the placebo bar are made entirely from natural food products and all ingredients present in the bars are of dietary origin and quality. The placebo bar is indistinguishable from the soy product in appearance, texture and taste and has a similar nutrient content. The nutrient composition and ingredients used in the supplement bars will be shown to you by the study coordinator. You will have the opportunity to sample the bar prior to beginning the study.

Laboratory Determinations: Blood samples will be analyzed for a number of hormones including estrone, estradiol, estrone sulfate, androstenedione, and Follicle Stimulating Hormone. Urine samples will be analyzed for phytoestrogens and lignans..

Blood Drawing: The total amount of blood taken for this study will be 150 ml (approximately tablespoons) for this study.

Risks: There are no known risks associated with consuming the soy bar or placebo bar. There may be other hormone related physiological symptoms that we cannot anticipate. Blood drawing, in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing when performed by somebody who has been specially trained and has experience in drawing blood decreases these risks. Because the soy bar and placebo contain milk products they may cause diarrhea or a stomach ache in individuals who can not digest or tolerate milk.

APPROVED: 06/05/95
VALID THROUGH: 12/13/94

Benefits: The data obtained in this study may be of no direct benefit to the participant but may yield insight into the effects of diet on hormone metabolism and its possible role in the cause and development of breast cancer. Data from the blood analyses will be available to you. The soy supplement may alleviate some symptoms such as hot flashes experienced during menopause.

Whom to Contact: If you have any questions about the study or experience any problems or injury or illness during it, you should call one of the following persons:

	Days	Evenings
Dr. Margo Woods	617-636-0809	617-646-6059
Dr. Sherwood L. Gorbach	617-956-5811	617-636-5111 beeper # 1193

Stipend: You will be paid $130 for participation in the study; $50 at the end of Phase I and the remaining $80 upon completion of the study. If you withdraw from the study before completing Phase I you will not receive any payment for your participation in the study. If you complete Phase I of the study but withdraw from the study before completing the study you will only be paid the $50.00 for completing Phase I. There will be no cost to the participant for participating in this study.

It is the policy of the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC) that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

APPROVED: 06/05/95
VALID THROUGH: 12/13/94

Consent Form 3 (Intervention)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

PARTICIPANT'S STATEMENT

I have read this consent form and have discussed with Dr._____ or his representative the procedures described above. I have been given the opportunity to ask questions, which have been answered to my satisfaction. I understand that any questions I might have asked will be answered verbally or, If I prefer, with a written statement understand that I will be informed of any new findings developed during the course of this research study.

I understand that my participation in this study is voluntary. I understand that I may refuse to participate in this study. I also understand that if, for any reason, I wish to discontinue my participation in this study at any time, I will be free to do so, and this will have no effect on my future care or treatment by my physicians or this hospital.

I understand that I am authorized all necessary medical care for injury or illness which is the proximate result of my participation in this research. Other than medical care that may be provided (and the stipend specifically stated in this consent form) there is no compensation available for my participation in this research study, however, I understand that this is not a waiver or release of my legal rights.

If I have any questions concerning my rights as a research subject in this study, I may contact the Human Investigation Review Committee at 617-636-7512. This office is located at 35 Kneeland Street, 6th floor Boston, MA 02111.

I have been fully informed of the above-described study with its risks and benefits, and I hereby consent to the procedures set forth above. I have received a copy of this consent form.

I understand that as a participant in this study my identity and my medical records and data relating to this research study will be kept confidential, except as required by law, and except for inspections by the study sponsor, United States Army Medical Research, Development, Acquisition and Logistics (USAMRDAL) Command (Provisional).

_____ _____

Participant Signature Date

_____

Participant name (printed) Address (street or PO Box, city, zip code)

APPROVED: 06/05/95
VALID THROUGH: 12/13/94

Consent Form 3 (Intervention)

Title: Menopausal Symptoms Study **Principal Investigator: Margo N. Woods, D.Sc.**

I have fully explained to _____ the nature and purpose of this above described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____
Principal Investigator/Representative

_____
Witness Signature

_____
Witness Printed name

_____
Date

APPROVED: 06/05/95
VALID THROUGH: 12/13/94

H. CONSENT FORM - SLOAN KETTERING

Memorial Hospital
1275 York Avenue
New York, NY 10021

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH
(Screening)

You are being asked to participate in a clinical research study. The doctors at Memorial Hospital study the nature of disease and attempt to develop improved methods of diagnosis and treatment. This is called clinical research. In order to decide whether or not you should agree to be part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent.

This consent form gives detailed information about the research study which the doctor and nurse will discuss with you. Once you understand the study, you will be asked to sign the form if you wish to participate. You will have a copy to keep as a record.

This research study is called: The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer

PURPOSE OF THE RESEARCH:

The purpose of this research study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

Menopausal symptoms have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms there are concerns about the affect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

Eligibility: To determine eligibility for this study you will be asked to complete a medical history questionnaire. If you are eligible and agree to participate in this study a screening blood sample will be taken (20 ml -approximately 2 tablespoons) and routine blood chemistries will be analyzed. You will be asked to complete a menopausal symptoms questionnaire. This screening will take approximately 2 hours. Based on the results of the questionnaire you will be assigned to either the

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control group or the intervention group. If you are assigned to the control group only baseline data will be collected as described in Consent Form 2; the length of this study is 3 days.. If you are assigned to the intervention group you will be asked to participate in a 7 month dietary intervention study as described in Consent Form 3.

Blood Drawing: The total amount of blood to be taken for the routine blood chemistry analyses will be 20 ml. (approximately 1 1/2 tablespoons).

Risks: The only risks associated with this part of the study are those associated with blood drawing, which in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing when performed by someone who has been specially trained and has experience in drawing blood decreases these risks.

Benefits: Although we hope that this research study will be of benefit to you, or that it will help others, we cannot say that it will help you directly. The data obtained in this study may yield insight into the effects of diet on hormone metabolism and its possible role in the cause and development of breast cancer.

Memorial Sloan-Kettering Cancer Center's Institutional Review Board is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected. It has reviewed this research study.

The clinicians in charge of this research study and the phone numbers where they can be reached are:

Dr. Ruby T. Senie 212-639-2373
Dr. Alexandra Heerdt 212-639-2471
Dr. Kimberly Van Zee 212-639-6997

If you need more information about this study before you decide to join, or at any other time, you may wish to contact one of these researchers. In the event you decide to partoicipate, they should also be called if there are any side effects from the research study. You may also call Janice Levy (212-639-5804) for information about the consent process, patient's rights or research-related injury.

This study has been funded by the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC). It is the policy of USAMRDALC that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

_____ _____
Participant's Initial Date

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PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH PARTICIPANT'S

TITLE

The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer.

PURPOSE

The purpose of this study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

STATEMENT OF RESEARCH TEAM MEMBER OBTAINING INFORMED CONSENT:

I have fully explained this research study to the patient _____ _____. In my judgement, and the patient's, there was sufficient access to information, including risks and benefits, to make an informed decision.

_____ _____

Date Signature of Research Team Member

_____

Name of Research Team member

PATIENT'S STATEMENT

I have read the description of the clinical research study or have had it translated into language I understand. I have also discussed the study with my doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it.

_____ _____

Chart Number Patient's Signature

_____

Patient's Name - Print

_____

Date

_____ _____

Participant's Initial Date

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Memorial Hospital
1275 York Avenue
New York, NY 10021

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH
(Intervention)

You are being asked to participate in a clinical research study. The doctors at Memorial Hospital study the nature of disease and attempt to develop improved methods of diagnosis and treatment. This is called clinical research. In order to decide whether or not you should agree to be part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent.

This consent form gives detailed information about the research study which the doctor and nurse will discuss with you. Once you understand the study, you will be asked to sign the form if you wish to participate. You will have a copy to keep as a record.

This research study is called: The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer.

The following information will describe the study and your role as a participants. Members of the research team will answer any questions you may have about this form and about the study. Please read this carefully and do not hesitate to ask anything about the information provided below.

Menopausal symptoms such as hot flashes and night sweats have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms there are concerns about the effect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

Purpose of the Study

The purpose of this study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

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PERM SHEET 8A

Description of Study Procedures

This 7 month dietary intervention study will use a random cross-over design to determine the effect of a **soy dietary supplement** on menopausal symptoms and endogenous hormones.

Based on the Menopausal Symptom Questionnaire you are eligible to participate in the Intervention Group for this study. This is because you are experiencing $\geq$ 5 hot flashes during daytime hours and/or $\geq$5 night sweats per week.

At baseline blood and urine collections will be done. Blood samples will be collected on two consecutive days (about 2 tablespoons or 30 ml each day) and a urine specimen will be collected. At the start of the study, women will be randomly assigned to either the soy supplement group or placebo group for three months. Subjects will consume two soy or placebo bars each day. At the end of the first three months blood and urine collections will be done as before. During the fourth month all study participants will consume placebo bars. During the remaining three months of the study women who were given the soy bar will be switched to the placebo bar and women who were given the placebo will be switched to the soy bar. At the end of the study blood and urine data will be collected as before.

Dietary data will be collected at baseline, and again at the end of three months and at the end of the study. At baseline a food frequency questionnaire (FFQ) will be completed; this takes approximately 20 minutes. A three day food record will be kept at baseline, and again during Phase I and Phase II. This requires that you write down all food and beverages consumed during a three day period. This takes approximately 10-20 minutes each day. You will receive instructions on how to keep a food record, and will be asked to review the completed food record with the study nutritionist. Throughout the study you will be asked to keep a daily diary of menopausal symptoms (hot flashes/night sweats).

Soy Dietary Supplement

The soy bar to be used in this study were developed by Scientific Hospital Supplies, Liverpool, UK and followed guidelines to develop a nutritional, high protein breakfast bar, made from soy.

Soy Bar Ingredients: soy extract (10 gm), linseed (1 gm), rolled oats, rice crispies, dried fruit, sugar syrup and dried milk.

Placebo Bar Ingredients: casein extract, corn oil, rolled oats, rice crispies, dried fruit, sugar syrup and dried milk.

The soy bar and placebo bar have the following nutrient content:

	Soy	Placebo
Calories (k cal)	130	120
Fat (gm)	4.2	4.0
Protein (gm)	6.5	4.0
Carbohydrate (gm)	17	17.0
Fiber (gm)	6.0	1.0

_____ _____

Participant's Initial Date

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All ingredients present in the bars are of dietary origin and quality. The bar is similar to toffee with has a crunchy covering. The product was found to be very desirable in taste tests. The placebo bar, made from is indistinguishable from the soy product in appearance, texture and taste and has a similar nutrient content. The soy supplement bar and the placebo bar are made entirely from natural food products.

Blood samples will be analyzed for a number of hormones including estrone, estradiol, estrone sulfate, androstenedione, and follicle stimulating hormone. Urine samples will be analyzed for phytoestrogens and lignans.

The total amount of blood taken will be about 10 tablespoons (180 ml) for this study.

Stipend

You will be compensated $130 for participation in the study; $50 at the end of Phase I and the remaining $80 at the end of the study.

Termination of Study

If you are unable to comply with the scheduled sequence for consumption of the dietary supplement and specimen collection, it may not be possible for you to continue in the study.

Side Effects

There are no known risks associated with consuming the soy bar or placebo bar.

Blood drawing by venipuncture, in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing performed by an experienced phlebotomist decreases these risks. If you are injured as a result of your participation in this research study, emergency care, hospitalization and outpatient care will be made available by the hospital and billed to you as part of your medical expenses. No money will be provided by the hospital as compensation for research-related injury.

If you are injured as a result of your participation in this research study, emergency care, hospitalization and outpatient care will be made available by the hospital and billed to you as part of your medical expenses. No money will be provided by the hospital as compensation for research-related injury.

Benefits

The data obtained in this study may be of no direct benefit to the participant but may yield insight into the effects of diet on hormone metabolism and its possible role in the cause of breast cancer. Data from the blood analyses will be available to you. The soy supplement may alleviate some symptoms (hot flashes/night sweats) experienced during menopause.

Financial Costs

There will be no charge for the analysis of urine specimens and bloods drawn for this study.

_____ _____

Participant's Initial Date

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Privacy

Your research and medical records are confidential. Your name or any other personal identifying information will not be used in any reports or publications resulting from the study. The Food and Drug Administration or other authorized agencies may inspect your records.

Right to refuse or withdraw

The choice to enter, or not enter, this study is yours. You are in a position to make a decision if you understand what has been explained and what you have read about the research. You may decide not to participate or you may wish to discontinue your participation in this study at any time. Either decision will have no effect on your future care or treatment by physicians of this hospital.

Compensation for illness or injury: In the event of a physical illness or injury resulting from your participation in this research study, no monetary compensation will be made, but any emergency treatment that may be necessary will be made available to you as part of your care.

Memorial Sloan-Kettering Cancer Center's Institutional Review Board is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected. It has reviewed this research study.

The clinicians in charge of this research study and the phone numbers where they can be reached are:

Dr. Ruby T. Senie	212-639-2373
Dr. Alexandra Heerdt	212-639-2471
Dr. Kimberly Van Zee	212-639-6997

If you need more information about this study before you decide to join, or at any other time, you may wish to contact one of these researchers. In the event you decide to participate, they should also be called if there are any side effects from the research study. You may also call Janice Levy (212-639-5804) for information about the consent process, patient's rights or research-related injury.

This study has been funded by the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC). It is the policy of USAMRDALC that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

_____ _____

Participant's Initial Date

95-39

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH PARTICIPANT'S

TITLE

The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer.

PURPOSE

The purpose of this study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

STATEMENT OF RESEARCH TEAM MEMBER OBTAINING INFORMED CONSENT:

I have fully explained this research study to the patient _____ _____. In my judgement, and the patient's, there was sufficient access to information, including risks and benefits, to make an informed decision.

_____ Date

_____ Signature of Research Team Member

_____ Name of Research Team member

PATIENT'S STATEMENT

I have read the description of the clinical research study or have had it translated into language I understand. I have also discussed the study with my doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it.

_____ Chart Number

_____ Patient's Signature

_____ Patient's Name - Print

_____ Date

March 1995

95-39

Memorial Hospital
1275 York Avenue
New York, NY 10021

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH
(Control)

You are being asked to participate in a clinical research study. The doctors at Memorial Hospital study the nature of disease and attempt to develop improved methods of diagnosis and treatment. This is called clinical research. In order to decide whether or not you should agree to be part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent.

This consent form gives detailed information about the research study which the doctor and nurse will discuss with you. Once you understand the study, you will be asked to sign the form if you wish to participate. You will have a copy to keep as a record.

This research study is called: The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer.

The following information will describe the study and your role as a participants. Members of the research team will answer any questions you may have about this form and about the study. Please read this carefully and do not hesitate to ask anything about the information provided below.

Menopausal symptoms such as hot flashes and night sweats have been associated with the decreased hormone levels that occur during menopause. Hormone replacement therapy (HRT) is given to approximately 30% of women in early menopause to alleviate some of the common symptoms experienced during menopause. While there are reported benefits of HRT on cardiovascular disease, osteoporosis, and menopausal symptoms there are concerns about the effect of HRT on breast cancer. Some studies have suggested that higher hormone levels may result in increased breast cancer risk.

Phytoestrogens are estrogen-like compounds found in plant products and high fiber foods including whole grains, legumes (especially soy) and some seeds. An increase in intake of soy products may contribute to the low incidence of breast and prostate cancer observed in Japanese women and men respectively. In addition, dietary soy has been shown to be inversely associated with breast cancer risk in Singapore. The relationship between soy and these cancers may be related to the phytoestrogens found in soy products, thus, it is possible that soy products may have an affect on hormone related, menopausal symptoms.

Purpose of the Study

The purpose of this study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

95-39

PERA
SHEE
8A

Description of Study Procedures

Based on the Menopausal Symptom Questionnaire you are eligible to participate in the Control Group for this study. This is because you are experiencing low levels of menopausal symptoms(<2 hot flashes during daytime hours or < 1 incidence of night sweats per week) or you have no menopausal symtoms. The term "Control Group" is used because the purpose of your participation as a control subject is to collect data (hormone levels in blood and urinary phytoestrogens) in order to establish baseline values in postmenopausal women with low or no menopausal symptoms. These baseline values will be compared with values of women who are experiencing frequent menopausal symptoms.

The length of the study for women in the Control Group is 3 days. Blood samples will be collected on two consecutive days (about 2 tablespoons or 30 ml each day) for hormone measurements. And during one day a 24-hour urine collection will be done. This requires that all urine voided during the entire 24 hours must be collected. You will also keep a daily diary of symptoms (hot flashes and/or night sweats) during these 3 days. In addition, dietary intake data will be collected using a food frequency questionnaire and a three day food record.

The total amount of blood taken will be about 4 tablespoons (60 ml) for this study.

Stipend You will be compensated $20 for your participation at the completion of the study.

Side Effects

Blood drawing by venipuncture, in rare cases results in an infection and occasionally in a black and blue mark. Blood drawing performed by an experienced phlebotomist decreases these risks. If you are injured as a result of your participation in this research study, emergency care, hospitalization and outpatient care will be made available by the hospital and billed to you as part of your medical expenses. No money will be provided by the hospital as compensation for research-related injury.

Benefits

The data obtained in this study may be of no direct benefit to the participant but may yield insight into the effects of diet on hormone metabolism and its possible role in the cause of breast cancer. Data from the blood analyses will be available to you. The soy supplement may alleviate some symptoms (hot flashes/night sweats) experienced during menopause.

Financial Costs

There will be no charge for the analysis of urine specimens and bloods drawn for this study.

Privacy

Your research and medical records are confidential. Your name or any other personal identifying information will not be used in any reports or publications resulting from the study. The Food and Drug Administration or other authorized agencies may inspect your records.

_____ _____

Participant's Initial Date

95-39

Right to refuse or withdraw

The choice to enter, or not enter, this study is yours. You are in a position to make a decision if you understand what has been explained and what you have read about the research. You may decide not to participate or you may wish to discontinue your participation in this study at any time. Either decision will have no effect on your future care or treatment by physicians of this hospital.

Compensation for illness or injury: In the event of a physical illness or injury resulting from your participation in this research study, no monetary compensation will be made, but any emergency treatment that may be necessary will be made available to you as part of your care.

Memorial Sloan-Kettering Cancer Center's Institutional Review Board is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected. It has reviewed this research study.

The clinicians in charge of this research study and the phone numbers where they can be reached are:

Dr. Ruby T. Senie	212-639-2373
Dr. Alexandra Heerdt	212-639-2471
Dr. Kimberly Van Zee	212-639-6997

If you need more information about this study before you decide to join, or at any other time, you may wish to contact one of these researchers. In the event you decide to participate, they should also be called if there are any side effects from the research study. You may also call Janice Levy (212-639-5804) for information about the consent process, patient's rights or research-related injury.

This study has been funded by the U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional) (USAMRDALC). It is the policy of USAMRDALC that data sheets are to be completed on all volunteers participating in research for entry into the USAMRDALC's Volunteer Registry Data Base. The information to be entered into the data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRDALC; and second, to ensure that the USAMRDALC can exercise its obligation to ensure that research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDALC for a minimum of 75 years. The computer data base will be kept confidential and not released to anyone.

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

TITLE

The Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormone Levels in Women at High Risk of Breast Cancer.

PURPOSE

The purpose of this study is to investigate the effect of dietary soy on hormones levels and menopausal symptoms in women at increased risk for breast cancer.

STATEMENT OF RESEARCH TEAM MEMBER OBTAINING INFORMED CONSENT:

I have fully explained this research study to the patient _____ _____. In my judgement, and the patient's, there was sufficient access to information, including risks and benefits, to make an informed decision.

_____ _____

Date Signature of Research Team Member

_____

Name of Research Team member

PATIENT'S STATEMENT

I have read the description of the clinical research study or have had it translated into language I understand. I have also discussed the study with my doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it.

_____ _____

Chart Number Patient's Signature

_____

Patient's Name - Print

_____

Date

95-39

I. RECRUITMENT MATERIAL - TUFTS

Date

Dear Ms.

Becuase you are a patient at the Breast Health Center at New England Medical Center Hospital we are writing to tell you about a study we are currently conducting on menopausal symptoms. This study is investigating the relationship between diet, hormones and menopausal symptoms.

You are eligible for the study if you are:

- ✓ post-menopausal
- ✓ not taking hormones
- ✓ not smoking
- ✓ at increased risk of breast cancer

The study is not very demanding but may ask you to:

- ✓ collect dietary data
- ✓ record menopausal symptom data
- ✓ provide blood samples for hormone determinations
- ✓ eat a dietary supplement bar twice a day

Enclosed is a flyer which provides more detailed information about the Menopausal Symptoms Study. If you are interested or would like more information about the study please call the Menopausal Symptoms Study Recruiting Line 617-636-6176. Ann LaBrode or Emily Potts will return your call and answer your questions.

Sincerely,

Susan Sajer, MD
Breast Health Center

Margo Woods, D.Sc.
Principal Investigator
Tufts University School of Medicine

MENPOP

MENOPAUSAL SYMPTOMS STUDY

We are looking at the relationship between diet, hormones and menopausal symptoms such as hot flashes. You may be eligible if you are:

- ✓ Menopausal
- ✓ 45-58 Years Old
- ✓ not taking hormones
- ✓ not smoking

For more information call 617-636-6176
Nutrition Unit, Tufts University School of Medicine

We are interested in having you join our study investigating the relationship between diet, hormones, and menopausal symptoms.

You may be eligible if you are:

- ✓ menopausal
- ✓ not taking hormones
- ✓ not smoking
- ✓ at increased risk of breast cancer

We may ask you to:

- ✓ collect dietary data
- ✓ collect menopausal symptoms data
- ✓ provide blood for hormone determinations
- ✓ take a tasty dietary supplement bar twice a day

You may benefit by obtaining diet and hormone information and a decrease of menopausal symptoms is possible. A stipend of up to $130 is available.

If you are interested or would like more information about the Menopausal Symptoms Study, please call the Menopausal Symptoms Study Recruiting Line at 617-636-6176. A study representative will return your call.

Menopausal Symptoms Study

Tufts University School of Medicine

We are interested in having you join our study investigating the relationship between diet, hormones and menopausal symptoms such as hot flashes and night sweats

You may be eligible if you are:

- ✓ **Post-menopausal**
- ✓ **Not taking hormones**
- ✓ **Not smoking**
- ✓ **Age 45-58**

If you are interested or would like more information about the Menopausal Symptoms Study, please call the Study Recruiting Line at 617-636-6176.

MENOPAUSAL SYMPTOMS STUDY

We are looking at the relationship between diet, hormones and menopausal symptoms such as hot flashes. You may be eligible if you are:

✓ Postmenopausal
✓ 45-58 Years old
✓ Not taking hormones
✓ Not smoking

For more information call 617-636-6176
Nutrition Unit, Tufts University School of Medicine

TELEPHONE SCREENING QUESTIONNAIRE
- MENOPAUSAL SYMPTOMS STUDY (MSS)

Complete entire questionnaire.

ELIGIBLE___
INELIGIBLE___
WILL BE ELIGIBLE___

Name: _____ Date_____

Address: _____

_____

Telephone: home_____ work_____

Are you currently a smoker? ___No ___Yes

Age_____ (48-58 eligible) Date of Birth_____

1. Are you post menopausal? ___No ___Yes, If Yes, was it:

___Natural
___Surgical, Were ovaries removed?
___No ___Don't Know ___Yes If yes,
___One, When_____
___Both When_____

2. When was your last menstrual period? Month _____Year_____ if < 1 year, not eligible
3. Have you had any menstrual bleeding in the past 12 months? ___No ___Yes

If yes, was it only spotting, ___No ___Yes,

4. Are you currently taking any hormone medications? ___No ___Yes
5. Have you taken hormone medications in the past? ___No ___Yes

If yes, when did you stop taking them? _____ **(< 6 months not eligible)**

Hot flashes, flushes and night sweats are sudden episodes of feeling warm, flushing, and /or sweating.

6. Do you ever have hot flashes? ___No **(Skip to Question 7)** ___Yes
   If yes, Do you have them during the day? ___No ___Yes
   How often, ___/Day ___/Week
   Do you have them at night? ___No ___Yes
   How often, ___/Day ___/Week
7. Are you currently taking any prescription medications? ___No ___Yes
   If yes, what are they? _____ Check medication list to see if patient still qualifies
   _____
   _____
   _____
8. Risk factors for Breast Cancer: (Use in Boston Only)

A. Do you have any relatives who have had breast cancer? ___No___Yes

If Yes, indicate:	Age of Diagnosis
___Mother	____
___Sister	____
___Daughter	____
___Other _____	____

B. Have you had a breast biopsy? ___No ___Yes, If Yes, how many? _____(number)

When did you have the biopsies?_____ What were the results? Benign___Malignant___

C. Have you had a breast biopsy diagnosed as atypical or proliferative breast disease? ___No ___Yes

MSS Control Checklist

ID.	SBV1 **CV1**	**CV2**
Forms		
Medical F3	❑ review	
Menopausal Sx F2	❑ review	
7 Day Sx Diary F5	❑ review	❑ review
Study Log F4		
Food frequency N2	❑ instruct	❑ review
3 Day Food Rec. N1	❑ instruct	❑ document
Consent Form S	❑ explain and sign	
Blood	❑ 40 ml	❑ 30 ml
Urine	❑ distribute kit	❑ Collect
Height/Weight	❑ Ht. ❑ Wt.	❑ Wt.
Schedule	CV2	
Calendar	CV2 urine coll 3 Day Food Rec 7 Day Sx Diary	
Give Ppt.	Calendar urine coll kit 3 Day food rec 7 Day Sx Diary	$20 payment

MSS Intervention Checklist

	SBV1	BV2	V3	V4	IV5	FV6	FV7
Forms							
Medical F3	❒review						
Menopausal Sx F2	❒ review		❒ fill out		❒ fill out		
7 Day Sx. Diary F5	❒ review ❒ distribute	❒ review	❒ reinstruct ❒ distribute	❒ review ❒ distribute	❒ review	❒ distribute	❒ review
Study Log F4		❒ instruct	❒ review	❒ review	❒ review	❒ review	
Food Frequency N2	❒ instruct	❒ review					
3 Day Food Rec.N1	❒ instruct	❒ document	❒ reinstruct ❒ distribute	❒ document ❒ distribute	❒ document	❒ distribute	❒ document
Consent Form S	❒explain/sign						
Supplement Bars		❒ 1 mo supply			❒ 1 mo supply		
Blood	❒ 40 ml	❒ 30 ml	❒ 30 ml	❒ 30 ml		❒ 30 ml	❒ 30 ml
Urine	❒distribute kit	❒collect sample	❒ distribute kit	❒ collect sample		❒ distribute kit	❒ collect sample
Height/Weight	❒ height ❒ weight	❒ weight	❒ weight	❒ weight	❒ weight	❒ weight	❒ weight
Schedule	BV2	V3 and V4	confirm V4	IV5	FV6 and FV7	confirm FV7	
Calendar	BV2 7 day Sx. diary 3 day food rec urine collect	mail study log V3 and V4 3 day food rec 7 day Sx. diary urine coll	V4 3 day food rec 7 day Sx. diary urine coll	IV5 3 day food rec 7 day Sx. Diary	FV6 amd FV7 3 day food recs 7 Day Sx diary Urine Coll Mail Study log	FV7 3 day food rec 7 day Sx diary Urine coll	
Give Ppt.	Calendar 7 day Sx. diary 3 day foodrec urine coll kit food frequency	Calendar Supp.Supply study logs mailers	Calendar 7 day sx diary urine coll kit 3 day food rec	Calendar 3 day food rec. 7 day sx diary $50 payment	Calendar Supp Supply study log Mailers	Calendar 3 day food rec 7 day sx diary Urine coll kit	Complete Evaluation $80 payment

J. RECRUITMENT MATERIAL - SLOAN KETTERING

THE BREAST SERVICE
DEPARTMENT OF SURGERY

Memorial Sloan-Kettering
Cancer Center

Patrick Borgen, MD
Chief, Breast Service

Hiram S. Cody, MD

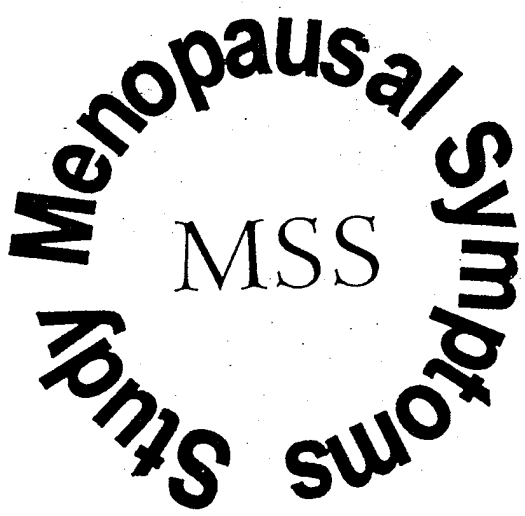
Alexandra Heerdt, MD

Jeanne Petrek, MD

Ruby T. Senie, PhD

Kimberly J. Van Zee, MD

MENOPAUSAL SYMPTOMS STUDY
212 639-2598



Memorial Sloan-Kettering Cancer Center
Breast Service, Department of Srugery
Menopausal Symptoms Study

212 639-2598

MENOPAUSAL SYMPTOMS STUDY

HORMONE LEVELS
&
SYMPTOMS

You may be interested if you are:

- menopausal
- 45 to 60 years of age
- not taking hormones
- not smoking

Memorial Sloan-Kettering Cancer Center
205 East 64th Street
New York, New York 10021

Breast Service
Department of Surgery
Menopausal Symptoms Study

212 639-2598

WHAT IS THIS STUDY ABOUT?

We are studying the relationship of hormone levels, food, and menopausal symptoms.

WHAT WILL I BE ASKED TO DO?

Keep a food record

Record menopausal symptoms

Provide blood and urine for hormone analysis

Some women will be asked to eat a tasty snack twice a day

WILL THERE BE ANY COST TO ME?

No, this study is funded by the Army Breast Cancer Research Program.

You will be given a small stipend to help cover parking and transportation costs.

HOW WILL I BENEFIT FROM THE STUDY ?

You will learn more about your hormone levels and menopausal symptoms.

FOR MORE INFORMATION
PLEASE CALL
PROJECT COORDINATOR

212 639-2598

CAN I BE IN THE STUDY?

We are interested in having you join our study if you fit the following description. Please call us at 212 639-2598 for more information.

*No menstrual period during past 12 months

*45 to 60 years of age

*Non-smoker

*Not taking hormones

*Family history of breast cancer

*No hysterectomy

June, 1995

Dear ,

Since you are a member of Memorial's Special Surveillance Breast Program, we would like to tell you about a study we are about to begin. Its purpose is to look at the relationship of hormone levels, menopausal symptoms, and nutrition.

If you can agree with each of the following statements you may be able to join this study:

I have had no menstrual period during the past year
My age is between 48 to 58
I am not taking hormones
A am non-smokers
I have a family history of breast cancer

During the study you may be asked to keep a three day food record, bring in a urine specimen, have blood drawn for hormone levels, describe any hot flashes, and eat a tasty food supplement bar.

For participating in the study you will receive a small stipend to cover travel and parking expenses and will receive information on your individual hormone levels. We have received research support from the federal government for this study.

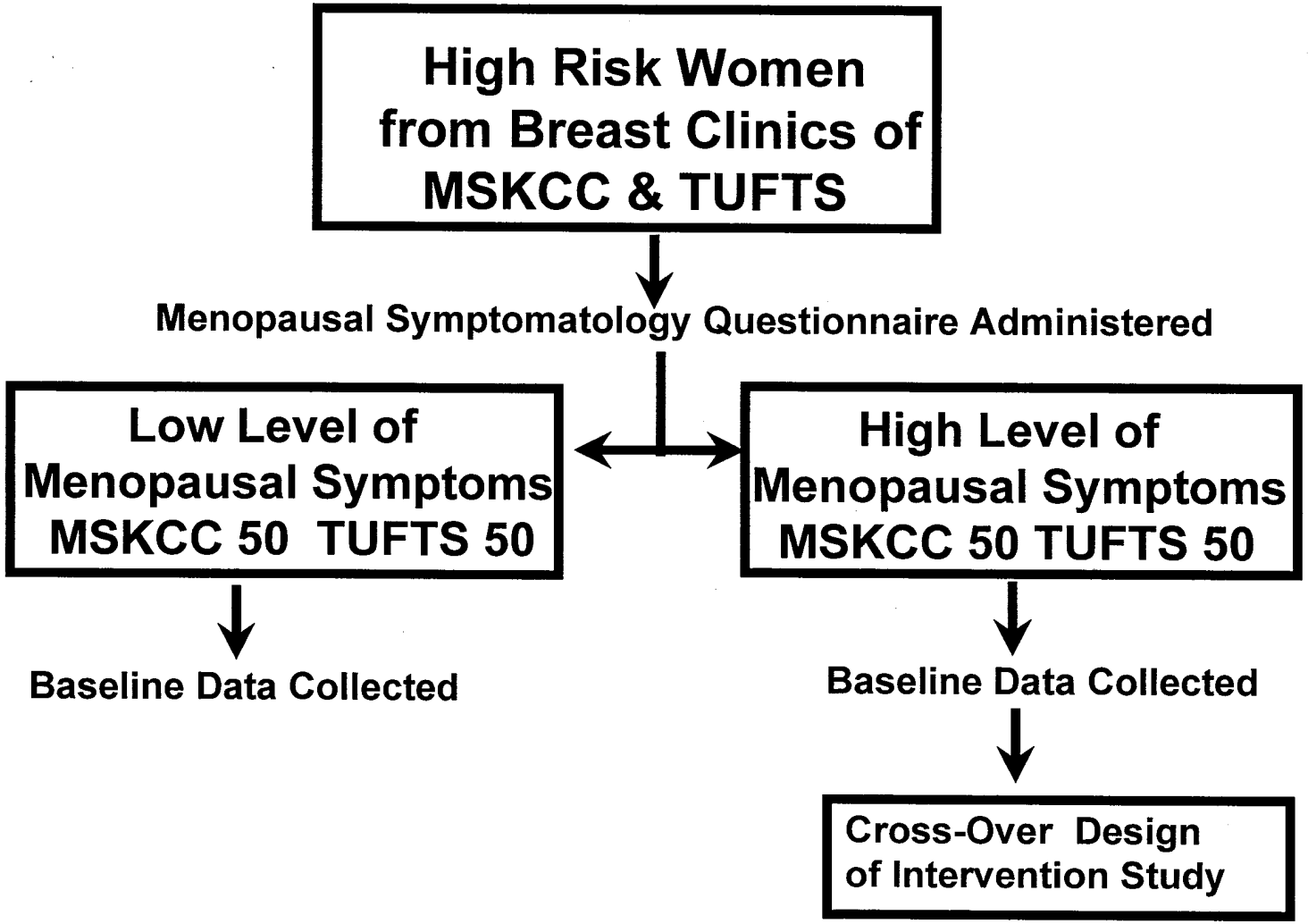
If you think you would like to be part of this study or would just like more information, please call Jane Fey, Project Coordinator of the Menopause Study at (212) 639-2598. Please leave a message and Jane will be glad to call you back to answer any of your questions.

Sincerely,

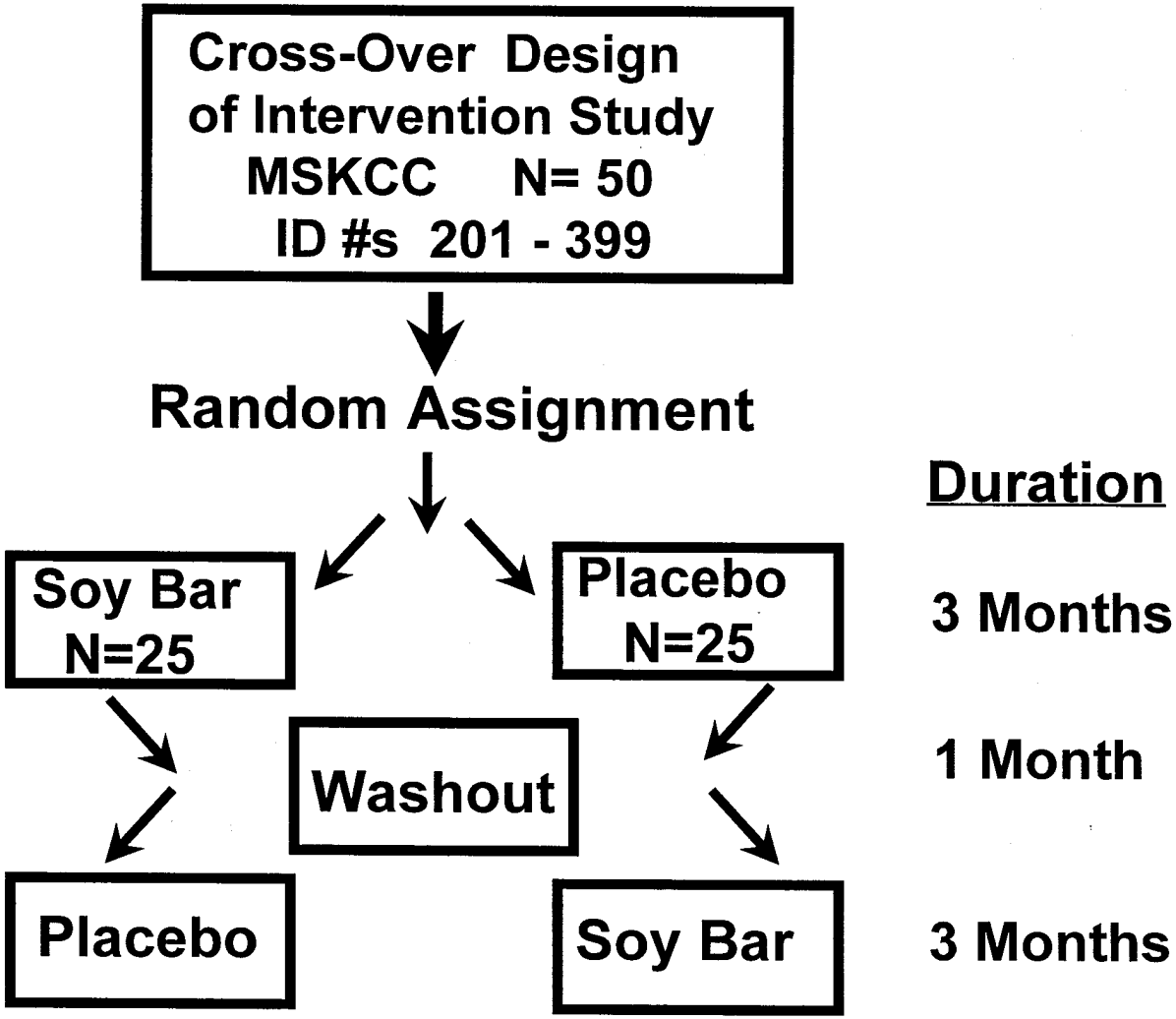
Alexandra Heerdt, M.D.

Kimberly Van Zee, M.D.

Menopausal Symptom Study
Assignment and Randomization

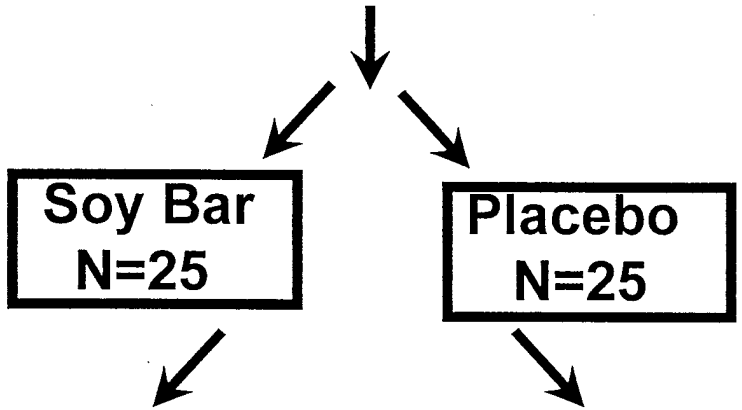


Menopausal Symptom Study
Assignment and Randomization
Memorial Sloan Kettering Cancer Center



Menopausal Symptom Study

Random Assignment - Tufts & MSKCC



Phase I

3 Months

Data Collection

Medical history & hormone use
Menopausal symptom questionnaire
Food frequency questionnaire
Blood & urine specimens collection

At enrollment & at end of Phase I

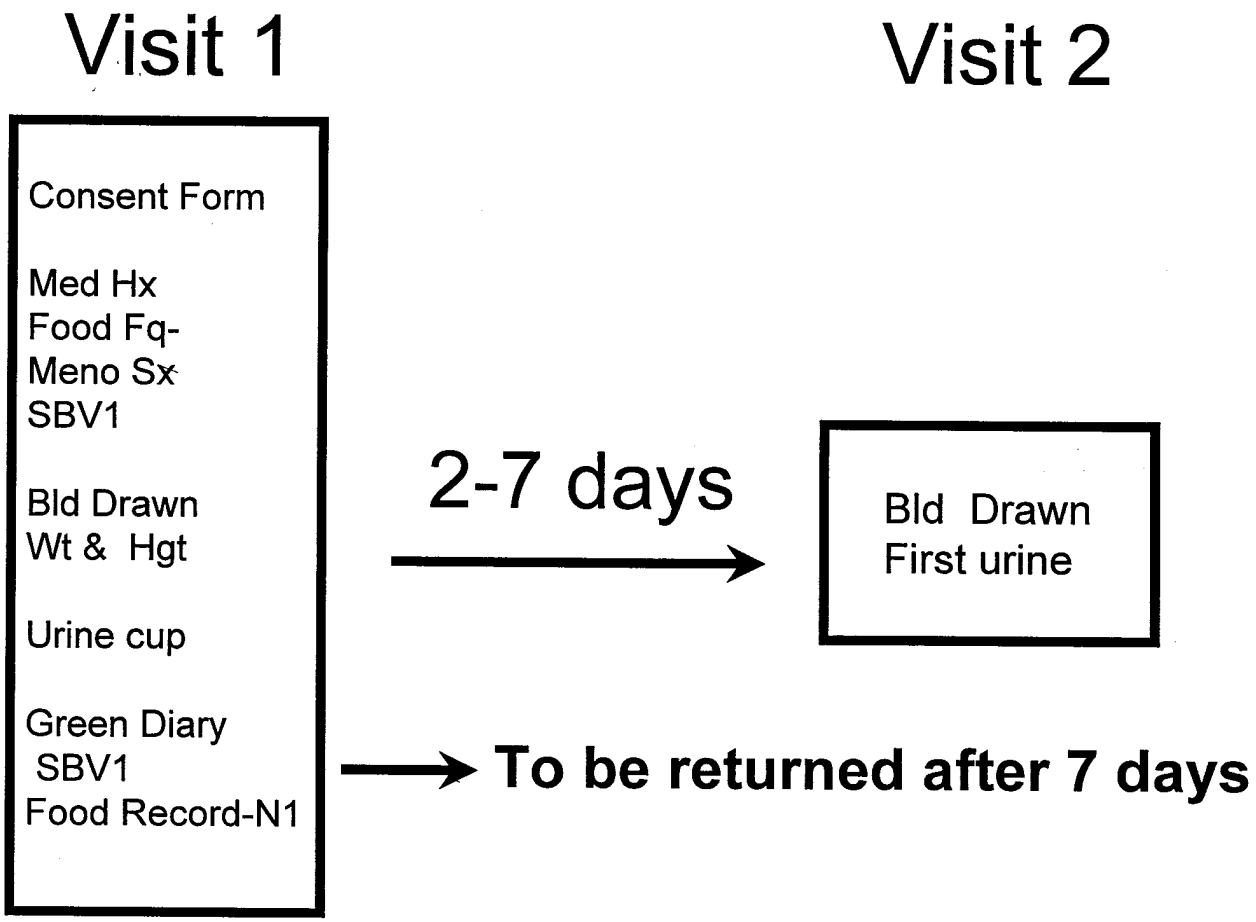
Coded dietary supplement provided

After random assignment

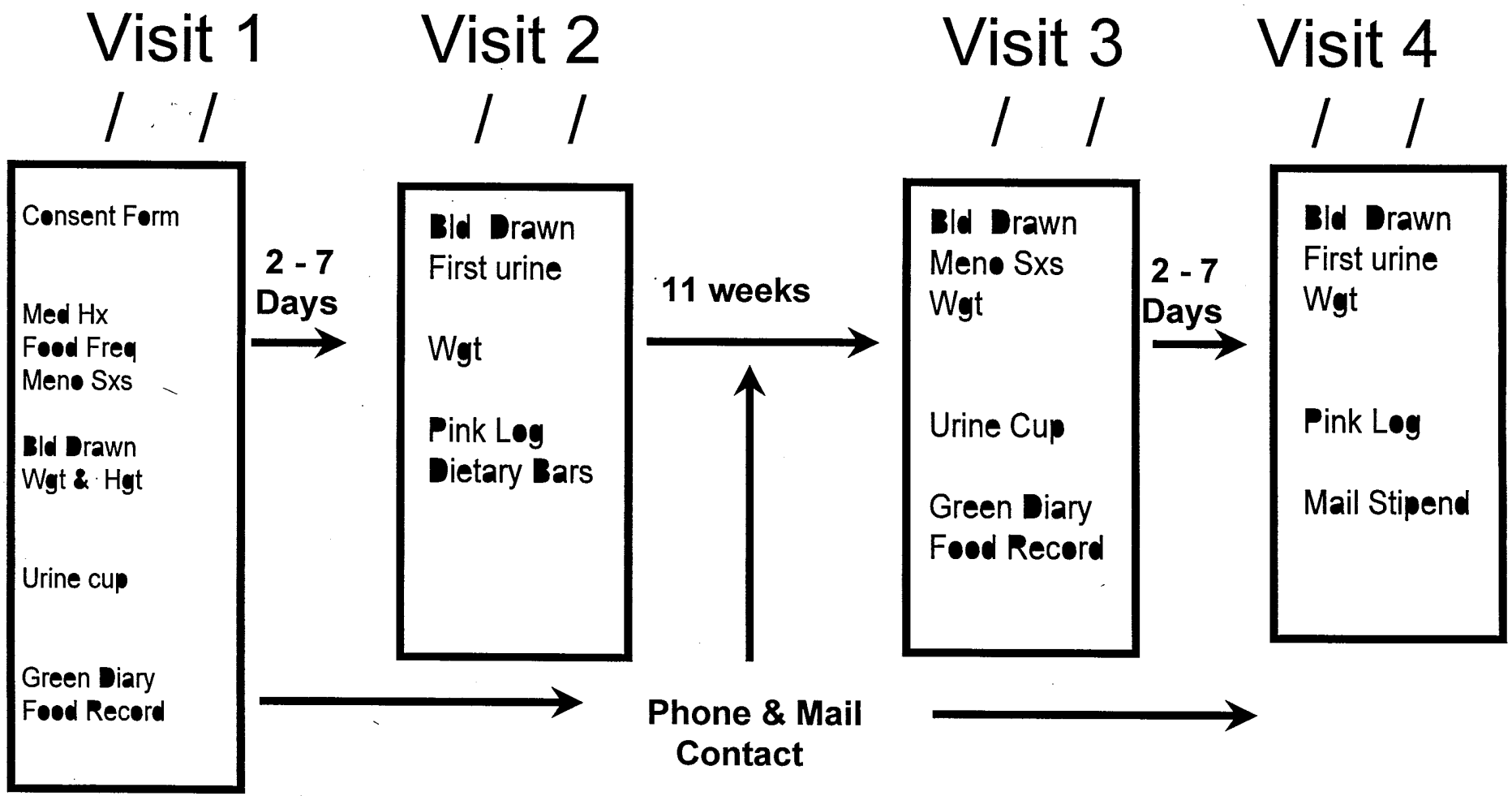
Brief questionnaire

Follow-up Phone Contact

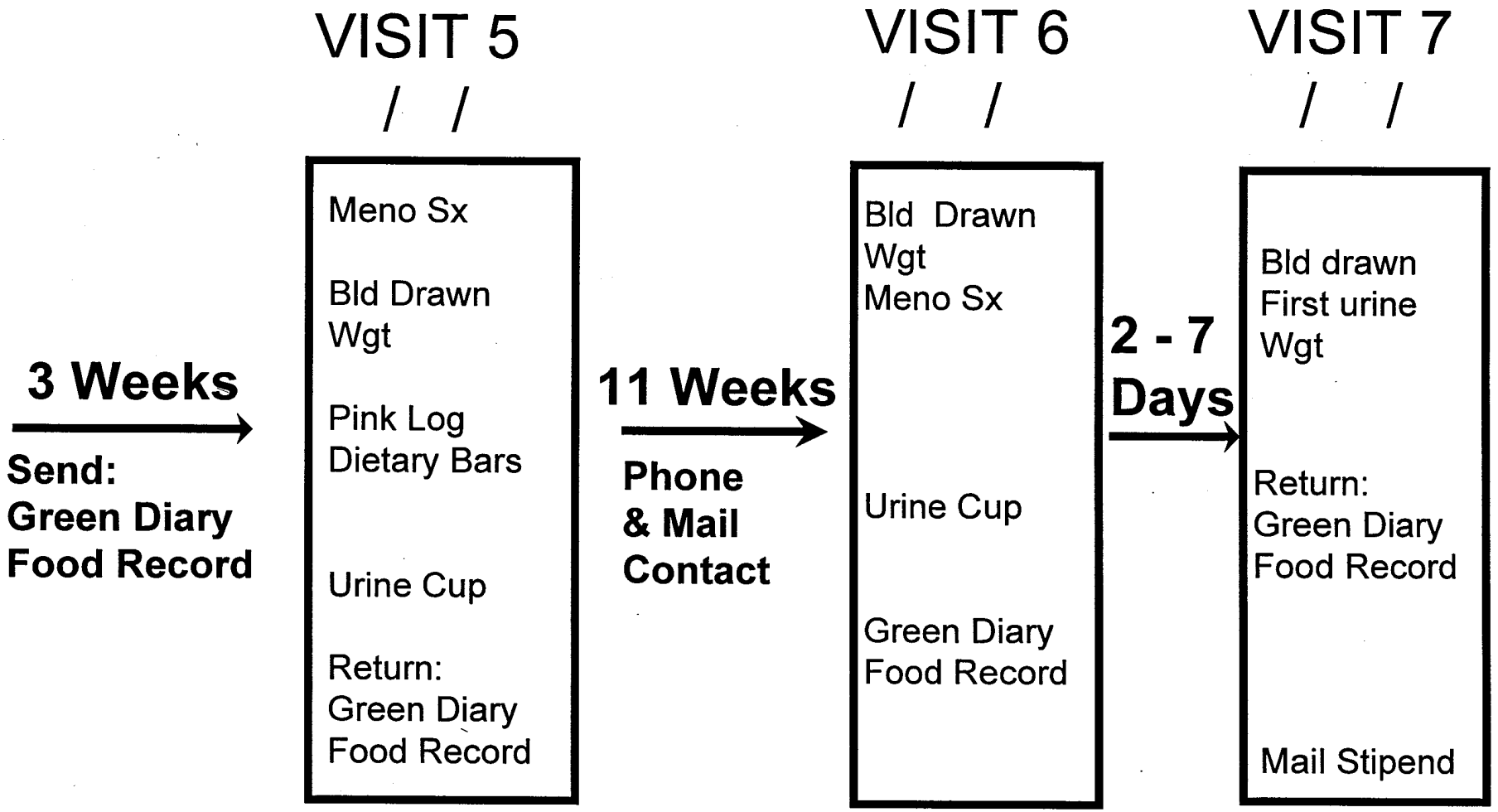
CONTROLS


Visit 1
Visit 2
Consent Form
Med Hx
Food Fq-
Meno Sx
SBV1
Bld Drawn
Wt & Hgt
Urine cup
Green Diary
SBV1
Food Record-N1
2-7 days
Bld Drawn
First urine
To be returned after 7 days

Dietary Supplement - Phase 1



Dietary Supplement - Resting & Phase 2



Menopausal
Symptom Study
7/95

K. PROTEIN TECHNOLOGY, INTERNATIONAL (PTI) SUPPLEMENT BAR NUTRIENT CONTENT

TUFTS UNIVERSITY - BARS - First Production

per serving/bar

	Units	Uncoated 675HG 2656-18-2	Uncoated Placebo 2656-18-3
Serving	g	56	56
Moisture	g	6.5	6.7
Fat	g	1.3	0.9
Protein	g	15.1	16.4
Ash	g	2.0	2.1
CHO (by diff)	g	31.1	29.9
Calories	Kcal	196	194
Ca	mg	711	497
Fe	mg	4.0	2.1
P	mg	502	304
Na	mg	168	106
Folic Acid	mcg	142	158
Pan Acid	mg	0.94	0.78
B6	mg	0.26	0.23
B2	mg	0.45	0.35
B1	mg	0.23	0.14
Vit A	IU	668	809
B12	meg	1.29	3.19
Vit C	mg	1.41	1.67
Vit D	IU	153	164