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CONTRACT NUMBER DAMD17-94-C-4120

TITLE: Effect of a Soy Dietary Supplement on Menopausal Symptoms
and Hormones in Women at High Risk of Breast Cancer

PRINCIPAL INVESTIGATOR: Margo N. Woods, D.Sc.

CONTRACTING ORGANIZATION: Tufts University School of Medicine
Boston, Massachusetts 02111

REPORT DATE: October 1997

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for public release;
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DTIC QUALITY INSPECTED 4

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19980206 034

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1997	3. REPORT TYPE AND DATES COVERED Annual (30 Sep 96 - 29 Sep 97)	
4. TITLE AND SUBTITLE Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormones in Women at High Risk of Breast Cancer			5. FUNDING NUMBERS DAMD17-94-C-4120	
6. AUTHOR(S) Margo N. Woods, D.Sc.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tufts University School of Medicine Boston, Massachusetts 02111			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) Women at high risk for breast cancer who are experiencing menopausal symptoms of hot flashes are being recruited for our study in Boston and surrounding areas. Breast Health Clinics and Women's Clinics are being used to inform women about the study. Radio advertising has also been used to locate our study population which has been highly successful. We have currently recruited 80 out of the 100 being sought. Women with ≥ 5 symptoms per day are randomized, using a double-blind, cross-over study design, to receive either a placebo bar or soy supplement bar for 3 months with one month of wash-out between protocols. Two blood samples are taken at baseline and during the last week of each of the two 3-month study periods. Serum levels of estrone, estradiol, free-estradiol, estrone-sulfate, androstenedione, luteinizing and follicle stimulating hormone are measured. Subjects keep a daily symptoms diary for the entire seven month study. A sub-set of women (N=10) will have serum genistein and diadzien analyzed to determine if their levels match their Asian counterparts. A matched group of control women with ≤ 2 hot flashes/day are currently being recruited. Fourteen have been recruited. This study proposes to determine: 1.) if a dietary soy supplement bar (containing 45 mg of phytoestrogens) relieves menopausal hot flash symptoms compared to a placebo bar, 2.) if a dietary soy supplement bar affects hormone levels, and 3.) the relationship between menopausal symptoms and hormone levels.				
14. SUBJECT TERMS Menopausal Symptoms, Hormones, High Risk Women, Soy Dietary Supplement, Phyestrogens, Diet, Breast Cancer, Clinical Trials				BER OF PAGES 13
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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1. INTRODUCTION

Phytoestrogens which are found in a number of fruits, vegetables, and grains, but occur in the highest concentrations in soy (1,2,3), have demonstrated both estrogenic and antiestrogenic activity (4). Although they are not steroids, structurally they resemble estrogens and they bind to estrogen receptors (5). Their estrogenic activity has been estimated at .002 and .001 (1/500 and 1/1,000) that of estradiol (6,7). Animal studies have reported a protective effect of phytoestrogens in mammary tumor models (8,9,10) while epidemiological studies in the Asian population have demonstrated an inverse relationship between soy intake and breast cancer (11,12,13). This has expanded the debate on identification of the environmental factors contributing to the observed lower risk of breast cancer in the Asian population to include intake of fat, fiber, and phytoestrogens (14,15).

These interesting effects of soy have prompted dietary soy studies in premenopausal women (16,17,18) measuring serum estrogens and gonadotropins and cycle length. A cross-cultural study on menopause (19) reported lower levels of hot flashes in Japanese women compared to women in North America which further implicated the possibility of soy intake in the Asian diet having biological estrogenic effects. It is estimated that in this population the daily intake of phytoestrogens is approximately 40 mg/day (20). Soy supplementation studies in post-menopausal women (21,22,23) have investigated serum hormone and gonadotropin levels, vaginal cytology, and menopausal symptoms.

Cassidy, et al. (16,17) studied premenopausal women who were given foods containing 45 mg of phytoestrogens/day for one month and reported an increase in estradiol in the follicular phase of the cycle, and a decrease in midcycle surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH). An increase in the length of the follicular phase of the menstrual cycle was also observed. Lu, et al. (18) also studied premenopausal women but gave foods containing 200 mg/d of phytoestrogens, for one month, and reported a decrease in estradiol. LH and FSH were not measured. The differences in estradiol responses observed between the two studies could be due to the difference in phytoestrogen intake since a biphasic response to phytoestrogens has been reported (24,25). Wilcox, et al (21) and Múrkies, et al (23), studied post-menopausal women consuming 45 g of soy flour/day (estimated phytoestrogen intake of 80-138 mg/d) for 2 weeks or 12 weeks respectively. Baird, et al (22), also studying post-menopausal women used foods containing 165 mg/day of phytoestrogens (in texturized soy vegetable protein) for one month and observed no change in estradiol, LH, or FSH. Both Wilcox, et al (21) and Baird, et al (22) reported changes in vaginal cytology that indicated an estrogenic effect of the soy products. Múrkies, et al (23) reported a decrease in menopausal symptoms in women while on the soy flour. However, a matched group consuming wheat flour also reported a decrease in menopausal symptoms, and there was no significant difference in reported hot flashes between the two groups after 12 weeks.

In order to test the effect of phytoestrogen intake on menopausal symptoms of hot flashes, we designed a double-blind study using a dietary supplement bar in which menopausal women consume 45 mg of phytoestrogens/day or a placebo containing casein protein with 0 mg of phytoestrogen. Participants are randomly assigned to consume either bar for 3 months with one month of wash-out,

followed by another 3 months of the alternate bar. Menopausal symptoms are tracked and recorded daily, and hormone and gonadotropins are determined at baseline and at the end of each dietary phase.

Primary study hypothesis: Consumption of 45 mg/day of phytoestrogens in a soy product will decrease the number of menopausal hot flashes reported by menopausal women with > 5 hot flashes/day and will decrease gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) after one month.

Secondary study hypotheses: Women reporting higher numbers of hot flashes (> 5/day) will have lower estrogen and higher FSH levels than women reporting lower numbers of hot flashes (< 2/day). Frequency of hot flashes will correlate to serum levels of estrogen and gonadotropins.

2. BODY

A. Experimental Methods

Study design: Intervention Group: Women reporting hot flashes are recruited into the study and during a one week baseline period record the number, time, and severity of each hot flash. Baseline blood is obtained to determine estrogen and gonadotropin levels. Those women who report, on average, 5 or more hot flashes per day are randomized into the double blind dietary intervention study in which they were asked to consume two dietary bars per day for a period of 3 months. One arm of the group consumes two dietary soy bars/day that contain 45 mg of phytoestrogens/day and the other arm consumed two dietary casein bars/day with < 1 mg of phytoestrogen/day. A one month washout period is followed by 3 months of the alternate dietary bar. During the last week of each 12 week study period two blood samples are obtained for determination of serum estrogens and gonadotropins. Women record daily hot flashes during the entire 7 months of the study.

Control Group: Women experiencing <2 hot flashes/day participate in a one week study to obtain information about hormones, dietary intake, and menopausal symptoms.

Subjects: Women, 45-59 years of age, at increased risk for breast cancer, who have not had a period for 6 or more months are recruited. Women currently taking hormones, steroids or other medications that interfere with hormone metabolism are excluded. Women who had previously taken hormones must be off them for at least six months prior to beginning the study. Women are excluded if they have undergone surgical menopause with no intact ovaries or currently have a high intake of soy products (greater than 2 times/week).

Dietary supplements: The soy supplement consists of a dietary bar that contains 196 kcal, 15.1 g protein, 1.3 g fat, and 24 mg of phytoestrogens (genistein and diadzien). The phytoestrogens are present in the soy protein fraction. The placebo bar uses casein protein in place of the soy protein and contains 194 kcal, 16.4 g protein, 0.9 g fat, and < 1 mg of phytoestrogens. The dietary bars are being supplied by Protein Technologies International. Subjects report whether they had consumed their two dietary bars each day as part of their daily recording of menopausal symptoms.

Menopausal symptoms: Subjects are given a menopausal symptom diary to keep daily records of the number of symptoms they have during the day and at night (night sweats). During the final week of each phase of the study, they are also asked to record the time of each hot flash and its severity (mild, moderate, or severe).

Hormone determinations: Blood samples are collected on two days, within one week of each other, after consuming the supplement bars for 11-12 weeks. Samples are centrifuged at 1400 x g rpm for 22 minutes at 4 C and the serum is stored at -70 C. Individual samples from each day are run in duplicate. An average of the two determinations is used in the analysis. Serum hormone measurements are carried out for estrone, estradiol, estrone-sulfate, androstenedione, testosterone, sex hormone binding globulin (SHBG), and free estradiol using radioimmunoassays involving solvent extraction and celite chromatography, as previously described (26,27). All samples are coded using a random number system and blinded duplicates were inserted as a quality control measure. LH and FSH are determined using a double-antibody radioimmunoassay utilizing a kit obtained from Radioassay Systems Laboratory, Inc., Carson, CA.

B. Progress Report/ Results

1. Site Visit Review

A site visit at Tufts University School of Medicine was carried out on October 9, 1996 for the contract "Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormones in Women at High Risk of Breast Cancer". The site visit team was composed of Drs. Hankinson, Kurzer, Rose and Kalish. Dr. Heydrick and Mr. Glisson were present to represent the review team and the Army. The two major recommendations of the review team were to terminate the sub-contract with Sloan-Kettering due to difficulty in reaching recruitment goals and extension of the contract into the fifth year to facilitate data analysis and writing of reports.

Following the recommendations of the committee, Tufts University School of Medicine canceled the sub-contract to Sloan-Kettering effective November 30, 1996. The budget for the grant was revised to reflect the changes and submitted to the Army contractors. This process was registered and approved. The other points of discussion from the review team will be discussed below, as appropriate.

2. Recruitment

A. Women reporting high levels of hot flashes (5 or more per day). Intervention Group.

During this current contract year, 74 women have been recruited into the study. Twenty-four women had been recruited into the study previously. Eighteen women dropped out of the study over the last year, resulting in a total of 80, out of the required 100 women that are being sought. Fifty-nine women are currently in the protocol. Twenty women are still needed to meet our recruitment goal of 100 and we are currently carrying out advertising and recruitment activities to complete this requirement. Since the dietary soy intervention is a seven month study, we have targeted the end of January to complete recruitment for the study. Over the past year, 250 study packets have been mailed to interested potential candidates for the study. We have had approximately a 30% response rate from these mailings which go to eligible persons that request further information. Currently, 10 packets have been mailed out and a followed-up call is made after two weeks.

Study recruitment data:

Current recruitment = 80

Number recruited prior to October, 1996 = 24 women

Number recruited between October 1, 1996 and September 30, 1997= 74

Number of drop-outs in the past year = 18

Current number who have completed the study = 24

Current number who are now in the study protocol = 59

Number needed to complete the study protocol = 20

Target date to reach this recruitment goal is January 31, 1998.

It is evident from these data that we have been successful in reaching and recruiting women in this age category, not taking HRT, and having 5 or more hot flashes per day.

Over the past year we have used 3 radio ads, 10 newspaper ads, attended 5 Health Conferences and posted the study on the Internet. We plan to continue to use radio spots, newspaper ads and health fairs to recruit this population since these methods have been the most successful over the past year.

B. Women reporting low levels of hot flashes (two or less per day). Control Group

The main focus of this past year has been to recruit women with 5 or more hot flashes/day to enter into the intervention protocol. Those who had 2 or less hot flashes per day, during the run-in period of one week, were approached to enter the Control group. This protocol only requires an additional week of recording of menopausal symptoms and two blood samples. However, no concerted effort has been directed yet at this study group. Currently, we have recruited fourteen women into this group. During the next year we will be carrying out a recruitment campaign to reach these women in order to complete the recruitment. This group is needed to address our secondary study hypothesis which seeks to confirm that women having more hot flashes will have lower serum estrogens and higher FSH values than women reporting lower number of symptoms. Since only two weeks of observation is required for this group, we believed they will not be difficult to recruit. While the control group may be less motivated to join the study, the short term requirements for this study protocol and the interest in obtaining their serum estrogen values will be sufficient incentive to recruit this population. In addition, those who are interested are offered a month's supply of the soy dietary bars.

Current number of women who have completed data for the Control Group = 14

3. Menopausal Status

The original grant contained requirements for eligibility that included a definition of "menopausal" as not having a period for at least one year and with a FSH/LH ratio greater than 1. During the course of recruiting, it became clear that the group of women who were having the most hot flashes were those in the "perimenopausal" phase. This appeared as a departure from what was implied in the literature (24,25) and was different than our previous experience with post-menopausal women in other studies. In order to recruit women who were having five or more hot-

flashes per day, the eligibility criteria was changed to "not having a period over the last 6 months". This would give us a population of menopausal and peri-menopausal women, but would still make it possible to answer the primary and secondary hypotheses of the study. An FSH/LH value of greater than 1 was still required. Higher baseline estrogens would be expected in the perimenopausal women, but since each woman served as her own control, this should not be important when comparing the soy versus the placebo dietary bar. Analysis of data on the correlation between number of hot flashes and baseline estrogens would need to control for time from menopause (or last period) in the analysis.

4. Risk Factors for Breast Cancer

Risk factors for breast cancer include: family history, fibrocystic disease, benign breast biopsies, breast cysts, and lobular carcinoma in situ (LCIS). Currently, 59 have family history, 25 fibrocystic disease, 16 benign breast biopsies, 12 breast cysts and 3 LCIS. Some participants have multiple risk factors. Eighteen women have none of the above, however, response to soy is not based on risk of disease and therefore the primary and secondary hypothesis can be addressed using all the participants. This topic was discussed with the site visit review team and everyone was in agreement that this would not present a problem.

5. Measurement of serum levels of phytoestrogens

Questions on the optimal dose of phytoestrogens to elicit various responses in the different organ systems (uterus, bone, cardiovascular, and hypocholesterolemic effects) were raised at the Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease in Brussels, September 1996 and at the Symposium on Phytoestrogen Research Methods in Tucson, September 1997. The question of quantification of intake to achieve a serum level equivalent to that found in Asian women, where soy is part of the habitual diet, was presented as a possible marker for many of the studies. In order to determine whether the intervention protocol is achieving this serum level, we have chosen serum from 10 women, at random, to be analyzed for serum phytoestrogen levels (genistein and diadzen). Baseline serum and serum while on the soy supplement will be analyzed for all ten women and in 3 women serum on the placebo will also be included. The determinations will be carried out in the laboratory of Dr. Herman Adlercreutz who is one of the recognized experts on phytoestrogens (26,27,28). He has determined and published the serum levels of phytoestrogens in Asian women (28). We have collaborated with Dr. Adlercreutz over the last 20 years on 5 or 6 projects involving estrogens and phytoestrogens. Additional funds will be sought for these analyses, if necessary since they will be critical in the evaluation of our data. Currently investigators are using 30-150 mg of phytoestrogen/day to study its effects in various sites (16,17,18,29,30,31). The site visit team enthusiastically supported the analysis of serum phytoestrogens in a sub-group of our study population.

6. Markers for calcium metabolism in our study population.

The site visit team enthusiastically supported the use of collected urine samples, from our study population, to determine calcium metabolism and the possible effect of soy supplementation. A number of urinary parameters have been used as indices of calcium metabolism. We are currently negotiating with two laboratories to carry out these determinations on a sub-set of the study

population to obtain some preliminary data and evaluate if further analysis are warranted. Currently effects of soy and phytoestrogens have been reported at levels of 90mg/day of phytoestrogen intake (32). At 45 mg/day, our study levels of phytoestrogen consumption, an effect may not be seen but verification would be desirable.

7. Data Entry

Data entry has been delayed due to the strong effort focused on recruitment. The data entry files have been developed on SPSS and data from the earlier group of 24 women has been entered. We have recently received the hormone and gonadotrophin data on this group of women also and will plan to enter this data into data sets already developed. Hormone samples are batched so that all samples from each woman are present which will eliminate any problem of batch variation within each subject. Since each woman serves as her own control in the comparison between placebo and soy effects on menopausal symptoms and hormone values, batching is of primary importance. This does delay the hormone determinations from the group of women recruited over the last year since many were recruited in the later part of the year and have not completed the 7 month protocol.

Plans are in place to begin data entry during the recruitment of the control group when demands of the intervention protocol will be greatly reduced. We are meeting regularly with our statistician, Dr. Donna Spiegelman, who has worked with us on numerous projects on diet and hormone and is well qualified to carry out the data analysis. A sub-contract to Dr. Spiegelman had been authorized last year.

8. Time Schedule

The site visit review team recommended a one year extension for the study due to the delay in recruitment and the termination of the second site at Sloan-Kettering. Currently we expect to need a six month extension but we would like to re-evaluate our options before the original ending of the granting period, October 31, 1998.

C. Future Plans/Conclusions

Plans for the current year, November 1997 thru October 1998, include completion of recruitment into the intervention group which we believe will be completed by January 1998. Therefore, all intervention subjects should complete the protocol by July 1998. Active recruitment of the control group is proceeding now and will intensify after January. Since this is only a two week protocol, we anticipate completion of this cohort by July or August.

Progress in data entry will depend on the difficulty in recruiting the final group of participants. If recruitment is easier than expected then data entry will progress throughout the year and may keep pace with availability of subject data. There is a two month lag time needed for hormone determination, so that the final hormone data can not be expected until August. Serum phytoestrogens and urinary markers for calcium metabolism will also be determined in a sub-group. Preliminary data analysis on the menopausal symptoms is expected for August-September. We will keep the funding office informed on our progress in case adjustments need to be made. A request for extension of the grant funding dates will be filed in an appropriate time schedule, if needed.

4. References:

1. Verdeal K, Ryan DS. 1979. Naturally-occurring estrogens in plant foodstuffs-a review. *J Food Prot.* 42: 577-583.
2. Setchell KDR. 1985. Naturally-occurring non-steroidal estrogens of dietary origin. In: McLaughlin JA, ed. *Estrogens in the environment.* New York: Elsevier, 69-85.
3. Eldridge AC. 1982. Determination of isoflavones in soybean flours, protein concentrates, and isolates. *J Agric Food Chem*, 30: 353-355.
4. Kaldas RS, Hughes Jr CL. 1989. Reproductive and general metabolic effects of phytoestrogens in mammals. *Reprod Toxicol.* 3:81-89.
5. Setchell KDR, Adlercreutz H. 1988. Mammalian lignans and phytoestrogens: recent studies on their formation, metabolism, and biological role in health and disease. In: Rowland JA. Ed. *The role of gut microflora in toxicity and cancer.* New York: Academic Press, 315-45.
6. Juniewicz PE, Morell P, Moses A, and Ewing LL. 1988. Identification of phytoestrogens in the urine of male dogs. *J. Steroid Biochem*, 31: 987-994.
7. Verdeal K, Brown RR, Richardson T, and Ryan DS. 1980. Affinity of phytoestrogens for estradiol-binding proteins and effect of coumestrol on growth of 7,12-dimethylbenz (α) anthracene-induced rat mammary tumors. *Journal of the National Cancer Institute* 64: 285-90.
8. Troll W, Wiesner R, Shellabarger CJ, Holtzman S. and Stone JP. 1980. Soybean diet lowers breast tumour incidence in irradiated rats. *Carcinogenesis* 1, 469-472.
9. Barnes S, Grubbs C, Setchell KDR, and Carlson J. 1990. Soybeans inhibit mammary tumor growth in models of breast cancer. In *mutagens and carcinogens in the diet.* Pages 239-253 [M.W. Pariza, editor]. New York: Wiley-Liss.
10. Hawrylewicz EJ, Huang HH, and Blair WH. 1991. Dietary soya bean isolate and methionine supplementation affect mammary tumour progression in rats. *Journal of Nutrition* 121: 1693-1698.
11. Nomura A, Henderson BE, and Lee J. 1978. Breast cancer and diet among the Japanese in Hawaii. *American Journal of Clinical Nutrition*, 31: 2020-2025.

12. Hirayama T. 1986. A large scale cohort study on cancer risks by diet - with special reference to the risk reducing effects of green-yellow vegetable consumption. In *Diet, Nutrition, and Cancer*, pp. 41-53. [Y. Hayashi, M. Nagao, T. Sugimura, S. Takayama, L. Tomatis, L.W. Wattenburg, and G.N. Wogan, editors]. Utrecht, The Netherlands: Tokyo/VNU Sci Press.
13. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, and Day NE. 1991. Dietary effects on breast cancer risk in Singapore. *Lancet* 337: 1197-1200.
14. Rose DP. 1986. Dietary factors and breast cancer. *Cancer Science*, 5: 671.
15. Rose DP. 1992. Dietary fiber, phytoestrogens, and breast cancer. *Nutrition*, 8:1:47.
16. Cassidy A, Bingham S, and Setchell K. 1994. Biological effects of isoflavones present in soy in premenopausal women: implications for the prevention of breast cancer. *American Journal of Clinical Nutrition*, 60:333-340.
17. Cassidy A, Bingham S, and Setchell K. 1995. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *British Journal of Nutrition*, 74: 587-601.
18. Lu L-J W, Anderson KE, Grady JJ, and Nagamani M. 1996. Effects of soya consumption for one month on steroid hormones in premenopausal women: implications for breast cancer risk reduction. *Cancer Epidemiology, Biomarkers & Prevention*, Vol 5:63-70.
19. Avis NE, Kaufert PA, Lock M, McKinlay SM, and Vass K. 1993. The evolution of menopausal symptoms. *Baillière's Clinical Endocrinology and Metabolism*, Vol. 7, No. 1: ISBN 0-7020-1697-7.
20. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa, T, and Okada H. 1991. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutri*, 54: 1093-1100.
21. Wilcox G, Wahlqvist ML, Burger HG, Medley G. 1990. Oestrogenic effects of plant foods in postmenopausal women. *Br Med J*. 301: 905-906.
22. Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KD, Weinberg CR, Haney AF, Wilcox AJ, and McLachlan JA. 1995. Dietary intervention study to assess estrogenicity of dietary soya among postmenopausal women. *J Clin Endocrinol & Metab*. 80:1685-90.

23. Múrkies AL, Lombard C, Strauss BJG, Wilcox G, Burger HG, and Morton MS. 1995. Dietary flour supplementation decreases post-menopausal hot flushes: Effect of soy and wheat. *Maturitas* 21: 189-195.
24. Yen SSC and Jaffe RB. *Reproductive Endocrinology Physiology, Pathophysiology, and Clinical Management*. Third Edition, W.B. Saunders Company.
25. Kronenberg F. 1990. Hot Flashes: Epidemiology and Physiology. Reprinted from *Multidisciplinary Perspectives on Menopause*, Volume 592 of the *Annals of the New York Academy of Sciences*, June 13, 1990, page 52.
26. Adlercreutz H, Fotsis T, Heikkinen R, Dwyer J, Goldin Br, Gorbach SL, Woods MN. 1982. Excretion of the lignans, enterolactone and enterodiols, and of equol in omnivorous and vegetarian postmenopausal women and in breast cancer. *Lancet*, 2:1295-1299.
27. Adlercreutz H, Honjo H, Higashi A, et al. 1991. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *American Journal of Clinical Nutrition*, 54:1093-1100.
28. Adlercreutz H, Gorbach S, Goldin B. 1992. Dietary phyto-oestrogens and the menopause in Japan. *Lancet*, 339:1233.
29. Petrakis NL and Barnes S. 1996. Abstract: Stimulatory effects of soy protein isolate on breast fluid secretion. *Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease*, Brussels, Belgium, 1996.
30. Lul L-J, Anderson KE, Nealon, and Nagamani. 1996. Abstract: Reductions in steroid and gastrointestinal hormone levels in men and premenopausal women with soya consumption for one month. *Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease*, Brussels, Belgium, 1996.
31. Kurzer MS, Zu X, Duncan AM, Merz BE, Glaspey A, Gaud K, Nagel T, and Phipps WR. 1996. Abstract: Effects of dietary soy isoflavones on estrogen action in premenopausal women. *Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease*, Brussels, Belgium, 1996.
32. Erdman JW, Jr., Stillman RJ, Lee KF, and Potter SM. 1996. Abstract: Short-term effects of soybean isoflavones on bone in postmenopausal women. *Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease*, Brussels, Belgium, 1996.