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Design and baseline characteristics of a trial on health effects of soy protein with isoflavones in postmenopausal women

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Abstract

This study is a double-blind, randomized, placebo-controlled trial to assess the effects of high amounts of phytoestrogens on bone mineral density, cardiovascular diseases, cognitive function, performance in activities of daily life and well being. Participants were healthy postmenopausal women, aged 60–75 years. Between March and September 2000, 202 women were recruited, and, after completion of the baseline measurements, randomized to either soy protein, containing 99 mg naturally occurring isoflavones or placebo (milk protein) daily for 1 year. Analysis of the endpoints will be based on the difference between baseline measurements and measurements at the end of the intervention period with group allocation as independent variable.

Keywords: Soy protein; Isoflavones; Health effects; Postmenopausal women

1. Introduction

Postmenopausal estrogen deficiency is held responsible for impaired well being with subsequent effects on cognitive function, increased risk of cardiovascular disease and increased fracture risk. There is evidence that postmenopausal hormone replacement therapy reduces fracture risk in elderly women [1–3]. Furthermore, a range of observational studies suggest beneficial effects of HRT on cardiovascular morbidity [4–6] and cognitive function [7–11]. However, conventional HRT is frequently associated with renewed vaginal bleeding, due to co-administration of progestagens in order to prevent endometrial cancer. Breast cancer risk is still increased [12,13]. The Heart and Estrogen/Progestin Replacement (HERS) study was not able to show cardiovascular benefit of opposed HRT in women known to have a heart disease [14]. This may well be due to attenuation of the estrogen effect by progestagens [15,16] or early harm due to increased thrombotic activity [17,18]. The estrogen/progestin treatment arm of The Women's Health Initiative trial was terminated early because of an increase in incidence of breast cancer in the inter-

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vention group and indications of an increased risk of cardiovascular diseases [19].

Estrogens in plant foods, the so-called phytoestrogens seem particularly interesting because of their tissue selectiveness. There are three main classes of phytoestrogens: isoflavones, coumestans and lignans. Phytoestrogens are known to improve well being and reduce perimenopausal discomfort, such as hot flushes [20]. There is limited evidence in humans that bone metabolism is affected favorably by the isoflavone derivative ipriflavone [21] and by soy isoflavones [22,23]. Studies in humans have shown that phytoestrogens decrease plasma lipids [24,25], LDL oxidation [26,27], arterial compliance [28] and endothelial cell damage [26]. These observations are strengthened by experimental data in atherosclerotic non-human primates, indicating that cardiovascular risk factors [29,30], coronary artery atherosclerosis [31], coronary vascular reactivity [32] and aortic cholesteryl ester content [30] are influenced favorably. Animal studies show that the reproductive system is not affected by phytoestrogens [33–35] and it has even been suggested that phytoestrogens are associated with decreased risk of endometrial cancer [36]. There are several studies that assessed direct relations between intake of soy products and breast cancer risk. Overall, protective effects are only observed for women consuming phytoestrogens at adolescence or at very high doses [37–39]. There is anyhow no evidence for an increased risk of breast cancer with consumption of phytoestrogens [40]. To determine whether phytoestrogens have beneficial effects on health in postmenopausal women without concomitant adverse effects on breast and endometrial tissue, we conducted a randomized double-blind trial. Naturally occurring isoflavones in soy protein were compared with milk protein as placebo.

2. Design and methods

The study is a randomized, double-blind, placebo-controlled trial to assess the effects of phytoestrogens, using soy protein, containing naturally occurring isoflavones, on well being, cognitive function, ability to perform activities of daily life (ADL) and bone mineral density (BMD; as a proxy of fracture risk) in elderly women. Furthermore, effects will be determined on endothelial function of the brachial artery (as a proxy of vascular aging) and urinary incontinence. As measures of safety, mammographical breast patterns (as a proxy of breast cancer risk) and endometrial thickness (as a proxy of endometrial cancer risk) will be assessed.

Between March and September 2000, 202 postmenopausal women underwent baseline measurements and were subsequently randomized to receive 99 mg of isoflavones in 25.6 g of soy protein or placebo (milk protein) daily for 1 year at one study site. The Institutional Review Board of the University Medical Center Utrecht approved the study protocol and all participants gave written informed consent.

2.1. Inclusion and exclusion criteria

Participants in our trial were healthy postmenopausal women aged 60–75 years, who lived in Utrecht or surroundings. The participants complied with the biannual call for participation in a national screening program for breast cancer in the year prior to the start of our trial, and a normal mammogram in the 12 months prior to enrolment in the study was required.

Women could not be enrolled when they had active liver or renal disease, a history of thromboembolism, history or presence of malignancy (except non-melanoma skin cancer). Furthermore, women who were current users of HRT or used HRT in the past 6 months or when they had lactose intolerance, a known allergy for milk protein or soy or endometrium thickness more than 4 mm could not be enrolled.

2.2. Recruitment

Recruitment started in March 2000, by written invitation of women participating in the national screening program for breast cancer. Women who were born between 1925 and 1940 and had a normal mammography in the year prior to enrolment were eligible and received a letter with information about the study. Those women who were interested returned an answering form and were called by one of the researchers to explain further details and answer questions. When women decided to participate, inclusion and exclusion criteria were checked during the telephone call. Next, an appointment was made to visit our outpatient unit.

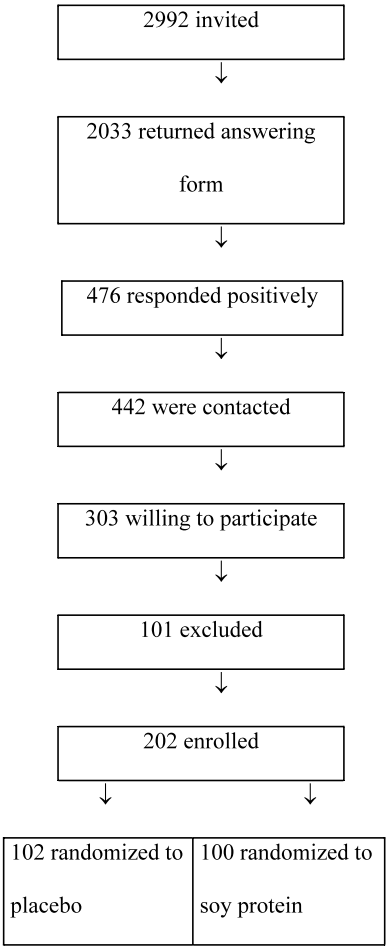

Fig. 1. Flow diagram of recruitment.

In total, 2992 women received a letter. Of the 2033 women who returned the form, 476 responded positively. Of those, 442 women were contacted, of whom 303 decided to participate. Of these 303 women, 101 were excluded (Fig. 1). Major reasons for exclusion were current use of HRT (26), a history of carcinoma (15) and a history of thrombosis (14). Finally, 202 women were enrolled during a 7-month period. Baseline characteristics of these women are shown in Table 1.

2.3. Screening visit

Potential participants were invited to visit the outpatient unit of the Julius Center for Health Sciences and Primary Care of the University Medical Center Utrecht, The Netherlands. After all remaining questions were answered, participants signed a written informed consent.

If no mammography in the year prior to the screening visit was available, an appointment for mammography was made at the department of radiology of the University Medical Center Utrecht. By means of a health questionnaire and measurement of endometrium thickness, we definitively determined whether subjects were eligible.

Table 1
Baseline characteristics of participants

Characteristic	Intervention	
	Soy protein (n=100)	Placebo (n=102)
	Mean (S.D.)	Mean (S.D.)
Mean age (years)	66.6 (4.8)	66.8 (4.7)
Height (cm)	164.3 (6.4)	163.8 (6.3)
Weight (kg)	71.3 (11.7)	69.7 (10.2)
Waist circumference (cm)	85.4 (11.4)	84.1 (8.6)
Hip circumference (cm)	103.0 (8.1)	102.5 (8.3)
BMI (kg/m^2)	26.4 (4.1)	26.0 (3.4)
Systolic blood pressure (mmHg)	138.5 (18.5)	142.0 (20.5)
Diastolic blood pressure (mmHg)	74.5 (11.5)	76.0 (13.5)
Age at menopause (years)	48 (6)	49 (4)
Years postmenopausal (years)	18.5 (7.5)	18.0 (6.0)
Fertile years (years)	34.5 (6.5)	35.5 (4.5)
BMD total hip (g/cm^2)	0.861 (0.112)	0.831 (0.119)
BMD lumbar spine (L1–L4) (g/cm^2)	0.919 (0.147)	0.892 (0.166)
	n (%)	n (%)
Ever use of estrogens	22 (22.2)[a]	23 (22.5)
History of fractures	33 (33.0)	28 (27.5)
Current smoking	19 (19.0)	13 (12.7)
Former smoking	33 (33.0)	34 (33.3)

[a] Unknown for one subject.

2.4. Randomization

After completion of the baseline tests, subjects were randomly assigned to the intervention or the placebo group in random blocks of 10. A list of randomization numbers was computer-generated by personnel not involved in the trial. Each random-

Table 2
Overview of the visits and measurements

	Baseline	3 months	6 months	9 months	Final visit
Inclusion and exclusion criteria	X				
Informed consent	X				
Endometrium thickness	X				X
Mammography (if necessary)	X				X
Health questionnaire	X				
Physical measurements	X	X	X	X	X
Ankle-arm-index	X				X
Brachial endothelial function	X				X
DEXA	X				X
Quality of life assessment	X				X
ADL assessment	X				X
Cognitive function	X				X
Dietary assessment	X	X	X		X
Adverse events		X	X	X	X
Compliance		X	X	X	X

ization number corresponded with one of the two possible interventions. Randomization numbers were assigned to the subjects in order of enrolment into the trial.

2.5. Intervention

The intervention consisted of 25.6 g soy protein containing 52 mg genistein, 41 mg daidzein and 6 mg glycetein (aglycone weights), as a powder (Solae^{TM} brand soy protein; DuPont Protein Technologies, St. Louis, MO). All three isoflavones occur naturally in soy. The total serving size of the product was 36.5 g. The powder could be mixed with foods or beverages. The placebo was an identically looking and tasting powder and consisted of milk protein (DuPont Protein Technologies, St. Louis, MO). The duration of the intervention was 12 months in which participants had to take the supplement on a daily basis. The dieticians provided the participants with the supplement and explained the possible applications of it. Participants also received a leaflet with recipes to get some ideas about preparing the supplement.

Personnel not involved in the trial fixed adhesives with randomization numbers to all the boxes containing the supplements. In this way, there was no chance of unblinding for any of the co-workers of the trial when providing the participants with new supplies.

If for one reason unblinding should be necessary during the course of the trial, a backup physician was available, who was informed about the trial but not involved.

2.6. Measurements

An overview of visits and measurements is shown in Tables 2 and 3, respectively. In total, women visited the outpatient clinic six times. Endpoints were assessed at baseline and after 52 weeks.

We measured height, without shoes, to the nearest of 0.5 cm and weight to the nearest of 0.5 kg. We also determined waist and hip circumference. Blood pressure and heart rate were assessed by a Critikon Dynamap on the right arm. Subjects were in sitting position and were still fasting. When the systolic or the diastolic blood pressure appeared to be high (>160 or >95 mmHg, respectively), blood pressure assessment was repeated after 5 min rest.

BMD was measured with dual energy X-ray absorptiometry (DEXA) using a Hologic QDR 1000 densitometer (Hologic, Inc., Waltham, MA). We measured BMD of the lumbar vertebrae L1–L4 and the left proximal femur. When women had a hip prosthesis on the left side, the right side was scanned. The scans were analyzed according to written manufacturer procedures.

We calculated the ankle-arm-index as a measure of generalized atherosclerosis [41]. Blood pressure at

Table 3
Overview of the different parts of the measurements

Domain	Measurement/test
Physical measurements	Height (m)
	Weight (kg)
	Waist circumference (cm)
	Hip circumference (cm)
	Blood pressure (mmHg)
Assessment of BMD	DEXA of the left proximal femur
	DEXA of the lumbar spine (L1–L4)
Cardiovascular measurements	Brachial endothelial function
	Ankle-arm-index
Quality of life assessment	Short Form of 36 questions (SF-36)
	Questions on Life Satisfaction^{Modules} (QLS^{M})
	Assessment of ADL
	HAQ
	Physical Activity Questionnaire for Elderly
	Physical Performance Test
	Handgrip strength
Cognitive function	
Memory	Rey Auditory Verbal Learning Test
	Immediate recall
	Delayed recall
	Recognition
	Digit Span (WAIS)
	Doors Test
Attention and concentration	Trailmaking Test
	Digit Symbol Substitution (WAIS)
Verbal skills	Verbal Fluency
	Boston Naming Test
Global cognitive function	Mini Mental State Examination
Depression	Geriatric Depression Scale
Intelligence	NLV (the Dutch version of NART)
Dietary Assessment	Semiquantitative food frequency questionnaire

the a. dorsalis pedis and the a. tibialis posterior was measured by using a Doppler device. Also, the blood pressure on both arms was assessed. For each leg, the highest of the two pressures was divided by the highest systolic pressure of both arms. In healthy persons, the index has to be 1 or slightly above 1.

Endothelial function was measured with B-mode ultrasound imaging in the brachial artery by assessing the artery diameter during reactive hyperemia (with increased flow leading to endothelium-dependent dilatation) [42,43].

With an ultrasound probe, the brachial artery is visualized. When a satisfactory longitudinal optimal image of the brachial artery is obtained, the position of the transducer is fixed. Three B-mode images showing the lumen diameter are frozen on the R-wave of EKG to provide information for off-line measurement of the 'baseline' lumen diameter for the ischemia test. Subsequently, a blood pressure cuff is inflated up to 50 mmHg above the participant's systolic blood pressure. After 4 min, the cuff is deflated. During the subsequent phase of reactive hyperemia, the image of the brachial artery is recorded every 15 s for 5 min. The images are digitalized and subsequently analyzed with special software [44]. This results in an absolute and a relative estimate of brachial dilatation during reactive hyperemia.

At baseline and at the end of the intervention period, quality of life was measured using the Short Form of 36 questions (SF-36) [45] and QLS^{M} [46].

We asked participants to fill in the forms at home 1 day before their visit at our outpatient clinic. On the

day of the visit, the research nurses checked the forms for missing answers or inconsistencies.

We used the Health Assessment Questionnaire (HAQ) [47,48], the questionnaire on mobility in elderly validated in elderly population [49] and the Physical Performance Test to measure performance in ADL [50].

Assessment of handgrip strength was at baseline performed in a random sample of 42 subjects and at final visit in a random sample of 88 subjects, due to the fact that interest in handgrip strength came up after the start of the study. Isometric grip force was measured with a Jamar hand dynamometer on the non-dominant hand. We asked the participants to perform the test twice, with a minimum of 30 s between the consecutive efforts. Both results were noted.

We tested several domains of cognitive function: memory (verbal (Rey Auditory Verbal Learning Test), visual (Doors Test), short-term and working memory (Digit Span)), visual attention and concentration (Digit Symbol Substitution Test; Trailmaking Test) and verbal skills (Boston Naming Test). We also asked participants to fill in the Geriatric Depression Scale on the day before their visit to our clinic. By means of this questionnaire, we are able to detect the presence of a vital depression, which can influence the performance on the cognitive tests.

Tests were administered by two neuropsychology students or by the investigators who were trained by experienced neuropsychologists. To avoid differences in the way tests were carried out, a standard procedure was agreed. Tests were done in a fixed order. Participants were tested individually in a quiet room between 8.30 A.M. and 12.30 P.M. The complete test battery took on average 1 h to be completed.

At baseline, fasting blood samples were collected. We measured fasting blood glucose immediately with a glucotouch meter. The samples were prepared by centrifugation and at each visit we stored 8 ml plasma and 6 ml serum for future testing at $-80\,^{\circ}C$.

After completion of the study, we determined lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and lipoprotein(a)), calcium, inorganic phosphorus, bone-specific alkaline phosphatase and insulin. All laboratory measurements were done in the SHO laboratory, Velp, The Netherlands. Baseline laboratory values are listed in Table 4.

Table 4
Baseline laboratory values

	Intervention	
	Soy protein (n=100)	Placebo (n=102)
	Mean (S.D.)	Mean (S.D.)
Calcium (mmol/l)	2.36 (0.10)	2.35 (0.08)
Phosphorus (mmol/l)	1.18 (0.15)	1.23 (0.13)
Ostase (μg/l)	12.54 (3.79)	13.03 (3.99)
Cholesterol (mmol/l)	6.19 (1.17)	6.12 (0.93)
HDL cholesterol (mmol/l)	1.54 (0.41)	1.51 (0.34)
LDL cholesterol (mmol/l)	4.16 (1.01)	4.09 (0.86)
Triglycerides (mmol/l)	1.33 (0.69)	1.40 (0.79)
Lipoprotein(a) (g/l)	0.33 (0.39)	0.27 (0.29)
Glucose (mmol/l)	5.5 (0.9)	5.4 (1.6)
Insulin (mU/l)	9.90 (6.26)	8.37 (3.93)

2.7. Dietary assessment

Measurements included a semi-quantitative food frequency questionnaire on habitual diet in the year prior to enrolment [51] that was slightly modified to capture dietary estrogen intake. The two-step dietary assessment comprised a simple self-administered questionnaire, followed by a structured interview with trained dieticians. From the questionnaire, the dieticians were able to calculate the habitual amount of protein intake. If necessary, the dieticians gave individual advice to compensate for the supplemental protein.

2.8. Adverse events

At each 3-month follow-up visit, participants were asked about possible adverse events. All diseases or conditions requiring medical attention were recorded.

Furthermore, participants were requested to notify the investigators of adverse events or diseases requiring medical treatment or hospitalization during the intervention period. Adverse events possibly related to the intervention included weight gain, gastrointestinal complaints and nausea.

2.9. Compliance

Compliance was monitored by means of supplement diaries. Subjects were asked to keep up a supplement diary during their participation, to record whether or

not they took the supplement, if they used it in one go or more and how they processed it.

We also asked our participants to count the remaining bags they had at home before each control visit, and at the end of participation subjects had to bring all spare bags.

We determined compliance in two ways, i.e. by calculating the mean daily sachet use first from the counted sachets and second from the supplement diary. After finalization of the study, serum genistein levels will be assessed in the final visit blood samples as an extra check on compliance.

2.10. Follow-up visits

During the intervention period of 12 months, subjects were asked to visit our clinic at 3, 6 and 9 months after randomization (Table 2).

At each follow-up visit, blood pressure was measured. To be aware of possible weight gain as a result of the intake of the supplement, we also measured weight every 3 months.

At 3 and 6 months, a fasting blood sample was taken. Again plasma and serum were stored for future testing.

To monitor food intake during the study, participants were asked to fill in food frequency questionnaires before each follow-up visit, except for the 9-month visit, asking about the former 3 months. This enables us to take into account potential changes to the habitual diet while taking the supplement. In case of problems with taking the supplement or in case of weight gain, the dieticians gave advice to our participants. Finally, they provided the participants with the supplement for the next 3 months.

The follow-up visits were also meant to encourage participants, assess compliance and to get information about possible adverse events.

2.11. Final visit

The final visit took place after 12 months of intervention. At the final visit, all tests carried out at baseline were repeated following the same procedures.

Participants who dropped out before 12 months but participated for at least 1 month were asked to come for a final visit.

2.12. Data analysis

Analysis of the endpoints will be based on the difference between the results at baseline and the results at final visit. We will use linear regression models with group allocation as independent variable to analyze the data. All statistical analyses will be carried out using the SPSS statistical package (SPSS, Inc., Chicago, IL), release 9.0. Analysis will first be done according to the intention-to-treat principle. We will also carry out per protocol analysis, because duration of isoflavone use might be an important factor.

3. Summary

Although animal studies have shown promising results about the protective role of isoflavones in several chronic diseases, data from studies in humans are scarce and with respect to bones and lipids contradictory. This might be due to the fact that most trials in humans were small and of short duration. For the present study, a double-blind randomized trial, we recruited 202 postmenopausal women for a 1-year intervention with soy protein containing naturally occurring isoflavones or placebo. First results can be expected in the coming months.

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