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Effect of whole soy and purified isoflavone daidzein on renal function—a 6-month randomized controlled trial in equol-producing postmenopausal women with prehypertension ☆

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ABSTRACT

Objectives: The aim of the study was to examine the long-term effect of commonly used whole soy foods (soy flour) and purified daidzein (one major isoflavone and the precursor of equol) on renal function among prehypertensive postmenopausal women who are also equol producers, a population most likely to benefit from soy intervention.

Design and methods: This was a 6-month double-blind, randomized, placebo-controlled trial. Two hundred seventy eligible Chinese women were randomized to either one of the three treatments: 40 g soy flour (whole soy group), 40 g low-fat milk powder + 63 mg daidzein (daidzein group) or 40 g low-fat milk powder (placebo group) daily each for 6 months. Fasting blood and 24-h urine samples were collected at the beginning and end of trial. Serum creatinine, cystatin C, urea, angiotensin-converting enzyme, minerals and 24-h urinary creatinine and minerals were analyzed. Estimated glomerular filtration rate (eGFR) was calculated with the Cockcroft–Gault and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.

Results: Two hundred fifty-three subjects completed the study according to the protocol. Urinary isoflavones indicated good compliance of participants. No significant changes were observed in most of renal parameters, however, there was a less decrease in $eGFR_{cockcroft}$ in 6-month change ($p = 0.044$) and %change ($p = 0.031$) with whole soy intake relative to milk placebo. Subgroup analysis among women with lowered renal function suggested whole soy consumption tended to improve markers of renal function relative to control.

Conclusions: Six-month consumption of whole soy tended to have a modest improvement of renal function in prehypertensive postmenopausal women with lowered renal function. Future trials in subjects with more declined renal function are necessary.

Trial registration: The trial was registered in ClinicalTrials.gov with identifier of NCT01270737. (URL: http://clinicaltrials.gov/ct2/show/NCT01270737)

Introduction

Chronic kidney disease (CKD) is a worldwide health problem that carries a substantial risk for both cardiovascular disease and all-cause mortality [1]. Elevated blood pressure (BP) and aging are important causes of CKD and associated with a premature decline in renal function even in individuals with normal kidney function [2,3]. Dietary factors play an important role in prevention and management of kidney function. Current evidence has shown that not only the quantity but also the types of dietary protein have important implications in renal disease [4]. Vegetarian diets have been studied as an essential means to halt progression of renal disease while maintaining adequate protein nutrition [5].

Soybeans provide high-quality plant protein and unique isoflavones (genistein and daidzein) associated with a potentially favorable effect on renal function [6]. In a variety of animal models of renal diseases, consumption of soy diet was renal protective by improving renal flow [7], reducing proteinuria [8] and renal histological damage [9], and resultantly retarding the development of kidney disease [10,11] than was casein or other animal protein. However, human studies on soy and renal function reported inconsistent findings. Single meal studies suggested that soy protein does not alter postprandial renal blood

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flow or glomerular filtration rates (GFR), whereas animal protein significantly increases these indexes. Several short-time (<8 weeks) clinical trials among patients with diabetes or CKD [4,12–16] indicated that substituting soy protein for animal protein is associated with less hyperfiltration and albuminuria, therefore slowing deterioration of renal function. However, the long-term effect of soy on renal function has not been adequately studied [5] and little evidence is available among postmenopausal women with prehypertension, a population with high risk of renal insufficiency due to aging and elevated BP. The National Kidney Foundation (NKF) has extensive recommendations on protein intake in patients with CKD [17]. However, the dietary preventive strategies to avoid CKD are not specified for persons at high risk or mild renal decline. In addition, most of previous clinical trials were of relatively short duration, involved a small number of participants and few such data are available in Asia.

Daidzein is the second major isoflavone (>40%) in soy and finally metabolized to equol by intestinal bacterial in 20–50% human adults. Setchell et al. [18] have hypothesized that equol production is the key to the clinical effectiveness of isoflavones, and equol producers derive greater benefits from soy supplementation than non-producers [19,20]. Thus, daidzein is postulated to be health beneficial at least in equol producers. Studies have suggested whole soy (less processed soy products such as soy milk, soy nuts, soy flour and tofu, etc.) intake are more effective than isolated soy component [21,22], and purified single isoflavone are more effective than complex isoflavones [23]. However, no RCT has tested the independent effects of daidzein on renal function and studies specifically designed among equol producers are limited.

This article reports the findings of whole soy (soy flour) and purified daidzein on renal function in a 6-month randomized controlled trial (RCT) among 270 pre- and early hypertensive Chinese postmenopausal women who are also equol producers. We tested the hypothesis that 6 months of intake of whole soy and purified daidzein would improve renal function.

Methods

Subjects recruitment

The study was a parallel-group, randomized, double-blind, placebo-controlled clinical trial to examine the effect of whole soy (soy flour) and isoflavones daidzein on cardiovascular risks and renal function. Subjects were recruited from the local communities mainly through advertisements in newspaper between December 2010 and January 2012. They were initially screened using a structured prescreening questionnaire by telephone interview and followed by a clinic visit to confirm their equol-producing phenotypes, in which subjects were required to consecutively ingest 63 mg daidzein for 7 days and collect 24-h urine for isoflavones testing.

This study was conducted according to the guidelines of Declaration of Helsinki approved by the institutional review boards of the Chinese University of Hong Kong. Written informed consent was obtained from each participant prior to enrolment.

Inclusion and exclusion criteria

The eligible subjects were Hong Kong Chinese women aged 48–65 years; at least 1 year menopausal; mean SBP 120–160 mm Hg or DBP above 80–100 mm Hg or both based on an average of four to six office BP readings at two occasions; equol producers which were defined as 24-h urinary log10 *S*-equol/daidzein ratio greater than –1.75 after daidzein challenge [24]. Subjects were excluded if they were currently or in preceding 6 months taking anti-hypertensive, hypoglycemic or weight reduction agents or on hormone therapy; had history of cardiovascular disease, untreated thyroid diseases, severe liver and renal dysfunction, or breast, uterine or ovarian cancer or other malignancies; known soy or milk allergy.

Study power

We enrolled a total of 270 subjects with 90 individuals in each study arm. The number was based on the conventional assumptions of two-sided 5% significance level. Assuming a withdrawal rate of 10%, we would have 80% power to detect a net change (mean difference) of 2.8 μmol/L in serum creatinine (SD of change 4.0 μmol/L), 0.07 mg/L in cystatin C (SD of change 0.08 mg/L) and an increase of 5.7 ml/min × 1.73 m^2 in estimated GFR by the Cockcroft–Gault formula ($eGFR_{cockcroft}$, SD of change 7.8).

Randomization and intervention

Block randomization in random block sizes of 6, 9 and 12 was conducted by a computer to generate a random list and each random number was corresponding to one of the three possible interventions. A total of 270 serial numbers were labeled on the identically looking boxes of supplements by personnel not involved in the trial and assigned to eligible subjects according to the sequence of their visits. All the research staff, technicians and subjects were blinded to the treatment codes. To assess the efficacy of blinding, the participants were asked at the end of the trial to guess their allocation. The proportions of participants who correctly guessed their allocations (7.8%, 7.8% and 6.5% for whole soy, daidzein and placebo groups respectively, with 128 women reported 'unknown' allocation) were not significantly differed among the three groups ($p = 0.947$), indicating the blinding was effective.

Treatment regimens and supplement preparation

Eligible women were randomized to one of the three groups: 40 g soy flour (whole soy group), 40 g low-fat milk powder + 63 mg daidzein (daidzein group), or 40 g low-fat milk powder (placebo group) per day each for 6 months. The nutrient profiles of three supplements were indicated in Supplementary Table 1. The supplements were concocted into beverage powders with similar color; odor and major nutrients profile by addition of minerals, starch or other food additives. The daily dose was filled into identical looking sachets. The isoflavones content in supplements were verified by a standard protocol [25]. The supplements were suggested to be mixed with 300 ml of water or beverages and partially replace breakfast or snacks. Subjects were required to discontinue the use of other dietary and herbal supplements, minimize the intake of soy foods (≤2 servings per week); refrain from high salt diet; restrict alcohol intake to ≤2 drinks per week and maintain their usual level of physical activity.

Data collection

Individual information was collected by trained interviewers by face-to-face interview based on structured questionnaires on socio-demographic data, medical history, medication, dietary habits and physical activities, etc. Dietary intakes over intervention were evaluated by a 3-day food diary which was completed by subjects at baseline and follow-up. Subjects received 30-min training on estimation of food amounts, portion and utensil sizes before food records. Dietary nutrients (energy, protein, total fat, calcium and soy isoflavones) were calculated based on the China Food Composition Table [26]. Participants were instructed to collect full 24-h urine on the day before the scheduled clinic visits. They were required to record the start and completion times of the sample collection, the total urine volume and the completeness of the urine collection. Urine samples were tested for isoflavones (genistein, daidzein and equol, etc.), electrolytes (sodium and potassium) and creatinine.

Overnight fasting (10–12 h) venous blood samples and 24-h urine samples were obtained at both baseline and end of the trial. Blood samples were taken immediately after urine collection and centrifuged at 3000 ×*g* for 15 min at 4 °C, and serum was isolated within 2 h after collection. Each subject's serum and urine samples were divided into several aliquots and stored at −85 °C until analysis.

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