Original Research

Estrogenic Effect of Yam Ingestion in Healthy Postmenopausal Women

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Key words: yam, *Dioscorea*, sex hormones, sex hormone binding globulin, estrogen metabolites, blood lipids, antioxidant, postmenopausal women

Objective: Yam (Dioscorea) has been used to treat menopausal symptom folklorically. This study was to investigate the effects of yam ingestion on lipids, antioxidant status, and sex hormones in postmenopausal women.

Methods: Twenty-four apparently healthy postmenopausal women were recruited to replace their staple food (rice for the most part) with 390 g of yam (*Dioscorea alata*) in 2 of 3 meals per day for 30 days and 22 completed the study. Fasting blood and first morning urine samples were collected before and after yam intervention for the analyses of blood lipids, sex hormones, urinary estrogen metabolites and oxidant stress biomarker. The design was a one arm, pre-post study. A similar study of postmenopausal women (n = 19) fed 240 g of sweet potato for 41 days was included as a control study. Serum levels of estrone, estradiol and SHBG were analyzed for this control group.

Results: After yam ingestion, there were significant increases in serum concentrations of estrone (26%), sex hormone binding globulin (SHBG) (9.5%), and near significant increase in estradiol (27%). No significant changes were observed in serum concentrations of dehydroepiandrosterone sulfate, androstenedione, testosterone, follicular stimulating hormone, and luteinizing hormone. Free androgen index estimated from the ratio of serum concentrations of total testosterone to SHBG decreased. Urinary concentrations of the genotoxic metabolite of estrogen, 16α-hydroxyestrone decreased significantly by 37%. Plasma cholesterol concentration decreased significantly by 5.9%. Lag time of low-density lipoprotein oxidation prolonged significantly by 5.8% and urinary isoprostane levels decreased significantly by 42%. For the control subjects fed with sweet potato, all three hormone parameters measured were not changed after intervention.

Conclusion: Although the exact mechanism is not clear, replacing two thirds of staple food with yam for 30 days improves the status of sex hormones, lipids, and antioxidants. These effects might reduce the risk of breast cancer and cardiovascular diseases in postmenopausal women.

INTRODUCTION

Since recent evidence from studies of Women's Health Initiative showed that the combined estrogen and progestine therapy increased risks of coronary heart disease, stroke, pulmonary embolism and breast cancer [1], an increasing number of postmenopausal women seek alternatives to ameliorate the undesirable conditions associated with decline of estrogens. Diosgenin (Fig. 1) from Mexican wild yam (*Dioscorea mexicana*) is one of the popular alternatives. Diosgenin was the main source of pharmaceutical corticosteroids and sex steroids during the 1950s [2]. There are over 600 species of *Dioscorea*, of which 12 are edible [3] and widely used as staple food or tonic food. Those grown as steroidal sources or medicinal herbs are bitter, not commonly eaten, and their liquid extracts are internally or externally used to treat asthma, intestinal spasm,

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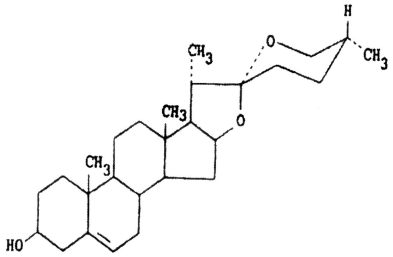

Fig. 1. Structure of diosgenin: the aglycon part of the yam steroid saponin.

rheumatic pain, menopausal symptom and menstrual disorder in native Americans and in traditional Chinese medicine [3–8]. Diosgenin is the possible active compound in *Dioscorea*. After oral administration, part of it is absorbed, distributed into liver, adrenals and walls of gastrointestinal tract, metabolized in the liver, and eliminated via the bile [9]. Two rodent studies indicated estrogenic actions of diosgenin. In ovariectomized mice, subcutaneous injection of 20–40 mg diosgenin/kg/d for 15 days stimulated the growth of mammary epithelium [10]. In ovariectomized rats with osteoporosis, sustained delivery of diosgenin by implanted capsules for 33 days reduced bone loss and reproductive tissue atrophy [11]. However clinical evidence of the efficacy of diosgenin was limited and no biochemical processes indicated that it could be transformed into sex hormones *in vivo*. A clinical trial in 1 men and 6 women aged 65–82 failed to demonstrate an effect of oral wild yam extract pills on serum dehydroepiandrosterone sulfate (DHEAS) levels [12], but the composition of this commercial yam extract was not cited. Another clinical survey did not find progestin bioactivity in the saliva of 11 women reporting consumption of Mexican yam products [13], but the kinds and amounts of yam products consumed and the menstrual status of each subject was not taken into consideration in that study. When yam (*Dioscorea villosa*) extract was used in the form of cream by topical application in healthy menopausal women, no effects on menopausal symptoms, lipids and sex hormones were found [14]. But topical application bypassed the effects of intestinal and hepatic metabolism, so the influence might be different from ingestion. Nevertheless, the ingestion of yam extracts was found to decrease plasma triglycerides, phospholipids, and increase HDL-C and antioxidant activity in the aged [12].

In Taiwan, yam is widely cultivated and used as tonic nourishment especially for postmenopausal women in recent years. Taiwanese yams consist of five major groups: D. alata, D. batatas, D. japonica, D. alata L. purpurea, and D. doryophora [15]. All are edible and some contain low levels of diosgenin (unpublished data from Lu TJ). Although the most common species of yam cited in herbal books is *Dioscorea villosa* [3], it is not commonly eaten. Feeding lyophilized Taiwanese yam powder improved upper gut function and the cholesterol profile in the plasma and liver of mice [16], and induced antioxidative effects in hyperhomocysteinemia rats [17]. To examine whether the folkloric belief that Dioscorea alleviates menopausal problems could be supported by scientific data, we recruited postmenopausal women to replace their daily staple food by fresh Taiwanese yam (*Dioscorea alata*), and explored its effects on lipids, antioxidants and sex hormone status. D. alata was used in this study because it was the most popular edible yam in Taiwan as well as in the world [3], and it did have a low level of diosgenin which might be dietarily significant.

MATERIALS AND METHODS

Subjects

Apparently healthy postmenopausal women were recruited from the community and local hospitals. Most of them lived nearby the university and had some kinds of leisure activities such as dancing, writing or painting in the campus. 35 enrolled, and after screening, 24 participated. All of them have heard of the health benefits of yam from medium and knew clearly the purpose of this study was to test and not to verify the folkloric belief. They were willing to receive the delicious and rather expensive yam dish, and make friends through this activity even without being paid. Inclusion criteria were as follows: 1. Amenorrheic for more than one year, 2. No hormone replacement therapy during the previous 6 months, 3. No regular consumption of yam (<2 times/week), 4. Aged 50–70 y, 5. Body mass index 22 ± 20%, 6. No history of cardiovascular diseases or diabetes. Written informed consent was obtained from each participant before inclusion in the study. The protocol was approved by the Human Experimentation Committee of Taiwan Adventist Hospital, Taipei, Taiwan.

Study Design

780 kg of yam cultivated in southern Taiwan was bought and stored under 15°C. The species of yam was identified by a specialist from Taiwan Agricultural Research Institute to be *Dioscorea alata*. After a 2-week run-in period, fresh and peeled yam cooked by various methods such as boiling, baking or frying in our kitchen was provided to subjects as breakfast and a dish of lunch in our dining room under the supervision of dietitians for 5 weeks, except on Sundays. So the total intervention duration was 30 days. During lunch, they also consumed their own regular food brought by themselves. The amount of yam consumed (390 g/d) was approximately adequate to replace staple foods in 2 of 3 meals per day. 390 g is approximately equal to the weight of 2 medium size potatoes, so it was not too much to be consumed. Volunteers were advised to maintain their usual life styles and diet habits and to keep their body weights unvaried. Two subjects withdrew from the study before completion. One could not get accustomed to ingesting the yam, and the other had to take antibiotics for a

long-term period, which might change the intestinal environment and the possible biotransformation of yam components, so we advised her to withdraw. Menopausal symptoms including hot flushes, vaginal dryness, night sweats, palpitations were monitored by questionnaires before and after yam intervention. The design was a one arm, pre-post study. Fasting blood and first morning urine samples were collected at the first morning of intervention and the morning following the end of intervention.

Subsequent to the study of yam, a similarly designed study with postmenopausal women fed with sweet potato was included as a control. None of the women has attended the yam trial. The subjects' serum hormones were measured for comparison with yam treatment. Sweet potato (Ipomoea batatas lam, Tai-Nong number 57) belonging to a Family different from yam, was an alternative staple in Taiwan for centuries. The amount of sweet potato consumed (240 g) per day was less than that of the yam study because of the less acceptable sweetness and the intervention duration was then extended to 41 days for sweet potato to get similar amount of calorie from sweet potato as that in the yam study. Serum samples from the sweet potato study were sent to our laboratory for estrone, estradiol and SHBG analyses.

Yam and Sweet Potato Analyses

General compositions and total dietary fiber of yam and sweet potato were determined using AOAC methods [18,19] (Table 1). Diosgenin content of yam was analyzed using HPLC [20]. Methanol extracts of freeze-dried powder of cooked yam were refluxed in 2 N HCl/methanol at 80°C for 2 h to give steroidal aglycones. After neutralization with NaOH, the solution was extracted with hexane 3 times. And then the extracts were washed with water 3 times. After evaporation of hexane, the residues were redissolved in methanol. Diosgenin in methanol was analyzed by HPLC utilizing a 4.6 × 250 mm Phenomonex column (5 μm) with acetonitrile/water (95:5) as the mobile phase. The column was eluted at a 1.0 mL/min flow rate and the absorbance was monitored at 203 nm.

Table 1. Composition of the Yam and Sweet Potato per 100 g Edible Portion

	Yam	Sweet potato
Energy (kcal)	102	121
Carbohydrate (g)	22.5	28.1
Crude protein (g)	2.8	2.0
Crude fat (g)	0.1	0.1
Moisture (g)	73.1	68.1
Dietary fiber (g)	1.3	2.4
Diosgenin (μg)	360	–

Nutritional Assessment

24-h recalls were used in three random unannounced days each for assessing dietary intake during run-in and yam intervention periods. A database for Taiwan food composition [21] was used to calculate the energy and nutrient intakes. Dietary fiber intake was assessed by a quick method developed by Marlett et al [22]. Body composition was measured using an 8-point tactile electrode, multifrequency, segmental bioelectrical impedance analyzer (SBIA, InBody 3.0, Biospace Co., Ltd., Seoul, Korea).

Blood and Urine Collections

After a 12-hour fast, blood was collected into two 10 ml-tubes, one containing EDTA (2.8 mg/mL of blood). Plasma or serum was separated from whole blood by centrifugation at 2000 g for 15 min and stored at −70°C under nitrogen. Early morning spot urine samples were collected in Vitamin C (1 mg/mL)-containing tubes and kept in ice before handing in. After centrifugation at 3000 g for 15 min, the clear supernatants were stored at −70°.

Biochemical Analyses

Samples from the same subject collected throughout the study were analyzed at the same time. Lipoproteins were separated from plasma by sequential ultracentrifugation [23] in NaBr density solution containing 10 μmol EDTA/L. Cholesterol and triglycerides of plasma and lipoproteins were measured by using enzymatic kits (Randox Lab., Antrim, UK). Low density lipoprotein (LDL) was oxidized in vitro by 10 μM copper and lag time of conjugated diene formation and amounts of LDL-TBARS produced after 2 h oxidation were measured as previously described [24]. Serum estrone, estradiol, DHEAS, androstenedione, testosterone, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicular stimulating hormone (FSH) were measured by enzyme immunoassay (EIA) kits (IBL, Hamburg, German) directly without extraction. When serum estrone was analyzed by EIA, low dilution overestimation was observed and the problem was overcome by using a more sensitive new kit (CAN-E-420, IBL, Hamburg, German) that required less serum volume. Competitive solid-phase EIA kits were used to measure urinary concentrations of an F_2-isoprostane, 8-iso-prostaglandin $F_{2\alpha}$ (Assay Designs, Ann Arbor, MI), and 2-hydroxyestrone (2-OHE_1) and 16α-hydroxyestrone (16α-OHE_1) (Immuna Care, Bethlehem, PA, USA) [25]. The concentrations are divided by the creatinine concentration to account for differences arising from variations in urine concentration. Urinary creatinine was determined by using a commercial kit (Randox Lab., Antrim, UK) after heated at 100°C for 5 min to destruct Vit C.

In the yam study, the intra-assay coefficients of variation (CV) for estrone, estradiol, DHEAS, androstenedione, testosterone, SHBG, LH, 2-OHE_1, and 16α-OHE_1 were 2.3, 1.7, 3.4,

1.1, 7.9, 3.5, 5.9, 1.6, and 1.6%, respectively, while inter-assay CVs were 9.0, 5.5, 3.8, 4.4, 7.3, 6.7, 8.6, 5.1, and 3.7%, respectively. In the sweet potato study, the intra-assay CV for estrone, estradiol and SHBG were 1.8, 2.1, and 1.9%, respectively, while inter-assay CVs were 9.0, 10.3, and 8.3%, respectively.

Statistical Analyses

Results were expressed in terms of means and standard deviations. Comparison between the values before and after intervention was made by a paired, two-tailed t-test. Data of serum and urinary hormones, urinary isoprostanes and LDL oxidation were not normally distributed as determined by Kolmogorov-Smirnov test, so the comparisons were made by a two-tailed Wilcoxon signed ranks test. For all measurements, results were considered statistically significant at $p < 0.05$. All statistical analyses were conducted by using SPSS 12.0.

RESULTS

General compositions and diosgenin content of yam used in this study were shown in Table 1. The content of diosgenin was much lower than that in the non-edible Mexican yam (Dioscorea mexicana) (5% wet wt of tuber) [26]. The characteristics of subjects were shown in Table 2A and Table 2B. Body composition and the percentages of calorie intake as carbohydrate, protein and fat were not changed before and after intervention (Table 2A).

The levels of serum estrone and SHBG increased significantly after subjects have been on yam diet for 30 days when compared with those before intervention (Table 3A). There were 6 serum samples from 3 women who had high SHBG levels after repetitive assay. Two of them reported to have treated and stable thyroid disease that was probably the reason to have high serum SHBG levels [27]. The SHBG data of these three subjects were excluded. The statistical significance in SHBG data still existed if the data of these three subjects were included (46.94 ± 60.58 vs 51.58 ± 67.82 nmol/L, n = 22, $p = 0.014$). The increase of serum estradiol did not reach a significant level ($p = 0.072$) (Table 3A), but its changes were positively correlated with the changes of serum estrone (r = 0.584, $p = 0.004$, n = 22), and were, as expected, inversely correlated with the changes of serum FSH (r = −0.488, $p = 0.018$, n = 22) and LH (r = −0.432, $p = 0.004$, n = 22) analyzed by spearman rank correlation. No significant difference was found in the serum levels of DHEAS, testosterone, FSH, LH and androstenedione after yam intervention (Table 3A). Free androgen index calculated as testosterone (nmol/L)/SHBG (nmol/L) ×100 [28] decreased (Table 3A). For those fed sweet potato, all three serum hormone parameters measured, estrone, estradiol and SHBG, were not changed after intervention (Table 3B). Urinary levels of 16α-OHE_1 and the sum of 2-OHE_1 and 16α-OHE_1 decreased significantly, but the ratio of 2-OHE_1 to 16α-OHE_1 did not change after yam intervention (Table 4).

Table 2A. Characteristics of Subjects and Daily Nutrient Intakes during the Yam Intervention

	Baseline	After or during yam
Age (y)	60.4 ± 6.8	–
Height (cm)	154.4 ± 3.9	–
Years since menopause	10.9 ± 7.7	–
Body weight (kg)	56.9 ± 7.4	56.1 ± 7.6
BMI (kg/m^2)	23.9 ± 3.5	23.9 ± 3.7
% body fat	30.8 ± 9.0	29.9 ± 9.0
Bone mass (kg)	2.2 ± 0.1	2.2 ± 0.1
Energy (kcal/d)	1378 ± 152	1358 ± 121
Carbohydrate (kcal/d)	748 ± 99 (54 en%)	754 ± 111 (56 en%)
Protein (kcal/d)	195 ± 41 (14 en%)	193 ± 23 (14 en%)
Fat (kcal/d)	435 ± 68 (32 en%)	412 ± 74 (30 en%)
Soy products (Servings/d)	0.28 ± 0.42	0.27 ± 0.29
Dietary fibers (g/d)	16.4 ± 2.5	17.8 ± 4.0

Values are means ± SD, n = 22. No significant differences were found.
1 serving of soy product: 100 g of tofu, 240 ml of soy milk, 25 g of wet soy milk skin, 70 g of dry tofu.

Table 2B. Age, Body Weight and Body Mass Index (BMI) at Baseline and after the Sweet Potato Diet

	Baseline	Final	*p*
Age (y)	54.6 ± 5.2		
Body weight (kg)	57.3 ± 5.6	56.8 ± 5.5	0.109
BMI (kg/m^2)	22.3 ± 1.9	22.1 ± 1.8	0.105

Values are means ± SD, n = 19.
Not significantly different from baseline by 2-tailed paired t test.

Table 3A. Serum Sex Hormone Concentrations at Baseline and after 30 days on the Yam Diet

	Baseline	Final	*p*
Estrone (pg/mL)	23.50 ± 7.77	29.57 ± 13.31*	0.003
Estradiol (pg/mL)	21.52 ± 10.05	27.28 ± 16.23	0.072
SHBG (nmol/L)	24.39 ± 11.97	26.69 ± 10.99*	0.019
DHEAS (μg/mL)	0.90 ± 0.43	0.94 ± 0.45	0.115
Testosterone (ng/mL)	0.51 ± 0.17	0.51 ± 0.19	0.768
FSH (mIU/mL)	51.03 ± 21.51	48.65 ± 20.47	0.175
LH (mIU/mL)	46.24 ± 24.72	49.52 ± 20.84	0.445
Androstendione (ng/mL)	2.39 ± 1.17	2.37 ± 1.19	0.755
Testosterone/SHBG (nmol/L/nmol/L) × 100	8.46 ± 3.92	7.22 ± 3.72*	0.022
(Estrone + estradiol)/SHBG (nmol/L/nmol/L) × 100	0.81 ± 0.40	0.91 ± 0.52	0.459

Values are means ± SD, n = 22, except in SHBG, Testosterone/SHBG, (Estrone + estradiol)/SHBG where n = 19.
* Significantly different from baseline by 2-tailed Wilcoxon Signed Ranks Test ($p < 0.05$).

Table 3B. Some Serum Sex Hormone Concentrations at Baseline and after the Sweet Potato Diet

	Baseline	Final	*p*
Estrone (pg/mL)	21.59 ± 10.35	26.16 ± 16.04	0.295
Estradiol (pg/mL)	19.26 ± 12.70	18.69 ± 12.77	0.629
SHBG (nmol/L)	40.00 ± 16.30	42.71 ± 24.44	0.445

Values are means ± SD, n = 19.
Not significantly different from baseline by 2-tailed Wilcoxon Signed Ranks test.

Plasma level of cholesterol decreased significantly, so did the levels of LDL-C and HDL-C but nonsignificantly (Table 5). The lag time of conjugated diene formation in LDL oxidized by copper was prolonged significantly (Table 6). Urinary excretion of isoprostane decreased highly significantly (Table 6).

Of the 22 subjects, only 2 had hot flushes at baseline, both of them reported to decrease the frequency of hot flushes at the end of intervention. 7 subjects had vaginal dryness at baseline, and 3 of them reported to improve.

DISCUSSION

Our study showed yam ingestion increased serum levels of estrone and SHBG (Table 3A). After the atrophy of ovary in postmenopausal women, estrone synthesized from adipose tissue becomes the major circulating estrogen. It possesses weak estrogenic activity, positively correlates with bone mass in postmenopausal women [29]. On the other hand, estrone [30] and estradiol, especially the free fraction, increased the risk of breast cancer [31]. The increase of free estrogen fraction was related to a reduction of SHBG and the protective effect of SHBG against breast cancer in postmenopausal women was demonstrated in several retrospective and prospective studies [32–34]. Therefore, the risk of breast cancer increased by estrogens might be balanced by the increased SHBG in this study where the ratio of estrone plus estradiol to SHBG did not increase after yam ingestion (Table 3A). Free testosterone, the biologically active testosterone, was associated with breast cancer [35] and cardiovascular diseases [36] in postmenopausal women. The estimated values of free testosterone decreased after yam ingestion and this beneficial effect appeared to be due to the increased levels of SHBG. Moreover, high SHBG levels were also shown to be protective against type 2 diabetes mellitus [37] and coronary heart diseases in women [38].

The outcomes of increased serum levels of estrone and SHBG (Table 3A) after yam ingestion were different from the other study [14] probably due to differences in yam species and administration methods. *Dioscorea alata*, the species we used, while not a significant source of diosgenin, is edible and most popularly consumed in Taiwan as well as in the world. The yam was administered by fresh form instead of yam extracts [12] and by ingestion instead of skin application [14]. The limitation of this study design, similar to other previous studies, is the lack of a control group or a crossover trial. Therefore, another study group in postmenopausal women fed with sweet potato, an unrelated staple food, devoid of diogenin and without any folkloric belief, was included to serve as a comparison group. This post hoc addition of a control still had some limitations such as that the two groups were not randomly derived from a group of women, therefore the average ages of the two groups were not the same. The other limitation was that the two interventions were not conducted in the same time of year (Apr vs. Sep). It has been observed that LH levels in Danish men was mildly but significantly affected by seasonal variation with peak levels in June–July [39]. As a subtropical island, the

Table 4. Urinary Excretion of 2-OHE_1 and 16α-OHE_1 at Baseline and after 30 days on the Yam Diet

	2-OHE_1	16α-OHE_1	2-OHE_1 + 16α-OHE_1	2/16αOHE_1
	ng/mg creatinine			ratio
Baseline	7.7 ± 8.3	8.2 ± 5.7	15.9 ± 13.1	1.15 ± 1.10
Final	4.6 ± 1.9	5.1 ± 1.8*	9.7 ± 3.0*	1.00 ± 0.50
p	0.192	0.001	0.050	0.468

Values are means ± SD, n = 20.
2-OHE_1 = 2-hydroxyestrone, 16α-OHE_1 = 16α-hydroxyestrone, 2/16αOHE_1 = ratio of 2-OHE_1 to 16α-OHE_1.
* Significantly different from baseline by 2-tailed Wilcoxon Signed Ranks ($p < 0.05$).

Table 5. Lipid Concentrations of Plasma and Lipoproteins at Baseline and after 30 days on the Yam Diet

	Baseline	Final	*p*
Plasma-TG (mg/dL)	116.1 ± 40.9	109.5 ± 39.5	0.379
Plasma-C (mg/dL)	204.8 ± 31.1	192.0 ± 29.6*	0.012
VLDL-C (mg/dL)	18.9 ± 11.1	17.6 ± 10.8	0.652
LDL-C (mg/dL)	115.8 ± 22.6	109.0 ± 24.2	0.075
HDL-C (mg/dL)	52.9 ± 17.9	50.9 ± 17.6	0.060
HDL_2-C (mg/dL)	36.3 ± 17.7	34.8 ± 17.5	0.091
HDL_3-C (mg/dL)	16.2 ± 2.1	15.7 ± 1.8	0.060
LDL-C/HDL-C	2.4 ± 0.7	2.3 ± 0.7	0.507

Values are means ± SD, n = 22.
TG = triglycerides, C = cholesterol, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein.
* Significantly different from baseline by two-tailed paired t test ($p < 0.05$).

Table 6. Lag Time of Conjugated Diene Formation, Thiobarbituric Acid Reactive Substances (TBARS) Production of LDL Oxidized by Copper and Urinary Isoprostane Excretion at Baseline and after 30 days on the Yam Diet

	Baseline	Final	*p*
Lag time (min)	62.3 ± 11.1	65.9 ± 9.6*	0.022
TBARS (nmol/mg protein)	56.0 ± 16.1	53.3 ± 16.4	0.475
Urinary isoprostanes (ng/mg creatinine)	9.85 ± 9.99	5.60 ± 7.09*	0.000

Values are means ± SD, n = 22.
* Significantly different from baseline by 2-tailed Wilcoxon Signed Ranks Test ($p < 0.05$).

climate in Taiwan is warm and changes in climatic air temperature and varieties of foods are very mild throughout a year. In addition, the duration of the intervention (5–6 weeks) was short, and the comparisons were made within groups, minimizing possible influences of seasonal variation. No changes in serum levels of estrone, estradiol and SHBG were found between pre- and post-sweet potato intervention (Table 3B). These results should help decrease the possibility that the observed changes in some of the biological endpoints might have been due to contributing factors other than yam, for instance, variability among biochemical analyses or just being in a study.

The estradiol levels measured in this study were somewhat higher (Table 3A), and made the ratio of estrone levels to estradiol levels close to 1. Most studies involving western or eastern populations showed estrone levels to be twice as much as the estradiol levels in postmenopausal women [40,41]. It was found that sex hormone values measured by direct radioimmunoassays without extraction were systematically higher than those obtained with organic solvent extraction, but within-batch reproducibilities of the subject rankings by relative levels were good [42]. In this study, the concentrations of serum sex hormones were measured by EIA without prior extraction. Samples from the same subject were applied not only in the same plate but also in the same column of the plate to assure exactly the same time interval in adding reagents by a eight channel pipette, so the values of the same subject before and after intervention should be comparable.

Estrogen metabolites, 4 and 16α-OHE_1, contribute to carcinogenesis for their potent estrogenic activity and their ability to bind to DNA, whereas, 2-OHE_1 is not [43]. Following yam ingestion, urinary 16α-OHE_1 and the sum of 2-OHE_1 and 16α-OHE_1 decreased compared with those at baseline (Table 4). Since 2 and 16α-OHE_1 comprise the major estrogen metabolites, this result might suggest that yam ingestion decreased the metabolism of estrogens and especially the formation of the carcinogenic metabolite, 16α-OHE_1. There was no significant change in the ratio of 2-OHE_1 to 16α-OHE_1 after yam ingestion (Table 4). The ratio of urinary or plasma 2-OHE_1 to 16α-OHE_1 has been suggested as a biomarker of breast cancer risk based on case control [44,45] and prospective [46,47] studies. On the other hand, some inconsistent findings have been reported from case-control studies [48], across-ethnic group surveys [49], and a recent large prospective study [50]. Therefore, the relationship between the ratio and breast cancer risk cannot be concluded yet.

Urinary F_2-isoprostanes, as a promising index of oxidative stress *in vivo*, mostly generated from the free radical catalyzed peroxidation of arachidonic acid [51], decreased very significantly after yam ingestion (Table 6). This result together with the prolonged lag time of LDL oxidation (Table 6) suggests a marked decrease of *in vivo* oxidant stress and an increase of antioxidant status.

Studies that examine the effect of the intake of isoflavones, the best known phytoestrogen, on plasma SHBG and estrone in postmenopausal women have previously been reported. The observations ranged from no to small increase in plasma SHBG [52–55] and from no influence to a decrease in plasma estrone (sulfate) [54,55] in their subjects. Isoflavone intake also decreases urinary 16α-OHE_1 [56], plasma cholesterol [57–59] and isoprostane [60] and prolongs lag time of *in vitro* LDL oxidation [56,57]. Therefore, the benefits of yam ingestion seem to be not less than soy ingestion in postmenopausal women especially for those who cannot tolerate the taste of soy but can consume yam as part of their staple food.

Factors that contribute to the increases in SHBG and estrone and the decrease in 16α-OHE_1 in this study are unclear. Whether diosgenin could be transformed into sex hormones *in vivo* or not, the diosgenin content of yam in this study was very low and the serum levels of estrone precursors, DHEAS, androstenedione and testosterone, did not increase significantly, therefore, diosgenin could not act as a precursor or activator of sex hormone syntheses in this study. Dietary phytochemicals such as indole-3-carbinol in cabbage [62], lignans in flaxseed [63] and soy isoflavones [56,64], and a low fat, high fiber dietary pattern [65] are effective in increasing the ratio of estrogen 2 to 16α-hydroxylation. Serum levels of SHBG were influenced by dietary fiber [66,67], calorie [68,69], fat [70], protein [69] and phytoestrogens [53], but the effects were not always consistent probably due to the existence of other confounding factors. In this study, the change of habitual diets was limited and was confined to the replacement of rice by yam. The intakes of soy foods, vegetables, fruits, meats, oils and cereals and dietary energy distributions were approximately the same before and during yam intervention as assayed in 3 random days each by 24-h recalls. The amount of dietary fibers was higher in yam than in rice (1.28 vs. 0.6 g/100 g), therefore the total daily dietary fiber intake increased about 2 g on yam diet. However, sweet potato contained more dietary fiber (2.4 g/100 g) than rice or yam, yet no increase in serum SHBG levels was found after sweet potato intervention. Therefore the effect of small increase of dietary fibers on SHBG levels was excluded. The other major controlling factor on SHBG level is insulin [71], which has been shown to decrease SHBG synthesis [72]. It is possible that the relative capacity to stimulate insulin is different between rice and yam. The published glycemic index of glutinous rice, sweat potato (Ipomoea batatas), and yam (*Dioscorea bulbifera*) are 140, 63, and 49, respectively [73]. Rice intake has been found to have positive association with SHBG levels in Chinese women in an ecological study [74]. Therefore, even though our data indicate the benefit of yam over rice in raising SHBG levels, we do not necessarily advocate against the consumption of rice. For the biological effects observed, it was possible that yam intake increased the hepatic synthesis of SHBG, and then increased the amount of SHBG-bound estrogens of which metabolic clearance decreased [75]. Consequently, the sum of major urinary estrone

metabolites decreased and the level of serum estrogens increased. The decreased urinary estrone metabolites after intervention could also be explained by a higher dietary fiber intake, which possibly increased the intestinal clearance of estrone metabolites [76]. There were few subjects with menopausal symptoms and the placebo effects were not excluded, so it was not possible to evaluate if yam intake relieved menopausal symptoms. Before we make conclusions about the estrogenic effect of yam, further studies are needed to explore the mechanisms that underlie the influences of yam.

CONCLUSIONS

Two third replacement of staple food with yam for 30 days increased serum estrone levels, which might benefit the declined estrogens in postmenopausal women. In the meantime, the increase in serum SHBG levels, the decreases in serum free androgen index, urinary 16α-hydroxyestrone, urinary isoprostane and plasma cholesterol levels, and the prolonged lag time of LDL oxidation, might potentially protect postmenopausal women against the risk of breast cancer and cardiovascular diseases.

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