British Journal of Nutrition (2010), **104**, 118–124
doi:10.1017/S0007114510000279

Daidzein-metabolising phenotypes in relation to serum lipids and uric acid in adults in Guangzhou, China

Kaiping Guo[1], Bo Zhang[1], Chaogang Chen[2], Shigeto Uchiyama[3], Tomomi Ueno[3], Yuming Chen[4] and Yixiang Su[1]*

[1]*Faculty of Nutrition, School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China*
[2]*Department of Clinical Nutrition, the Second Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*
[3]*Otsuka Pharmaceutical Company Limited, Japan*
[4]*Faculty of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China*

(Received 6 July 2009 – Revised 14 January 2010 – Accepted 16 January 2010 – First published online 8 March 2010)

Previous studies have suggested that daidzein's metabolites, equol and *O*-desmethylangolensin (*O*-DMA), rather than daidzein itself may contribute to the beneficial effects of soya foods in the prevention of CVD. The present study aims to assess the proportion of equol and *O*-DMA producers, and to compare differences in anthropometric factors, serum lipids, glucose and uric acid between producers and non-producers in Chinese adults aged 20–69 years. For the present cross-sectional study, 202 subjects (100 women and 102 men) were recruited. Twenty-four-hour urinary daidzein and its metabolites were determined in these subjects while on their usual diet and again after a 3-d isoflavone challenge. Fasting serum lipids, glucose and uric acid were examined on their usual diet. Three days of 24 h dietary recalls were used to assess dietary intakes. Of the 202 subjects, 27 (13·4 %) and 27 (13·4 %) excreted equol and *O*-DMA on their usual diet, and 101 (50 %) and 94 (46·5 %) produced equol and *O*-DMA after a load of 80 mg/d isoflavones. Equol producers showed lower serum uric acid (−10·2 %, $P=0·001$), TAG (−29·5 %, $P=0·007$) and waist:hip ratio (−2·6 %, $P=0·032$), and tended to have higher HDL cholesterol (6·3 %, $P=0·069$) compared with equol non-producers. There were no significant differences in serum lipids, glucose and uric acid between *O*-DMA producers and non-producers. In conclusion, equol phenotypes might influence cardiovascular risk.

Isoflavones: Equol: *O*-desmethylangolensin: Blood lipids: Uric acid

Soya isoflavones, mainly daidzein and genistein, have a similar chemical structure that is similar to that of the oestrogens[1], and they consequently bind to oestrogen receptors to exert either oestrogenic or anti-oestrogenic activity[2]. Many studies indicate that higher soya intake is associated with a lower incidence of CVD[3,4]. However, the effects of soya isoflavones are inconsistent in human studies. One possible explanation for variant responsiveness to isoflavones may be differences in the metabolism of isoflavones among individuals, specifically variation in equol and/or *O*-desmethylangolensin (*O*-DMA)-synthesising capacity.

Equol is a gut bacterial metabolite of daidzein, and it has been proposed to be an important bioactive metabolite because of its selective binding to oestrogen receptor-β[5–7]. Certain distinct intestinal bacteria are capable of metabolising daidzein to equol and/or *O*-DMA. Previous studies have shown a substantial variance in the ability of individuals to synthesise equol or *O*-DMA[8,9]. Typically, 20–30 % of adults produce equol and 80–90 % of them produce *O*-DMA in response to a soya food or isoflavone challenge in Western populations[10–11]. Asians were more likely to be equol producers than Caucasians[12,13]. No study has yet assessed the proportion of equol and *O*-DMA producers in a Chinese population.

It has been suggested that two subpopulations – equol producers and non-producers – respond differentially to intervention with soya or isoflavones[14]. Some small studies found that *O*-DMA production was associated with higher bone mineral density[15,16]. However, no study has yet examined if the equol or *O*-DMA producers have lower cardiovascular risk in Chinese populations. The present study aims to assess the frequency of equol and *O*-DMA excretion in Chinese adults on their usual diet and after a load of 80 mg/d isoflavones for 3 d, and to examine the relationship between the ability of equol and/or *O*-DMA production and some cardiovascular risk factors.

Experimental methods

Subjects and study procedure

Community-based apparently healthy subjects aged 20–69 years were recruited in Guangzhou, China, by intensive

Abbreviation: *O*-DMA, *O*-desmethylangolensin.

* **Corresponding author:** Professor Yixiang Su, fax + 86 20 8733 3166, email suyx@mail.sysu.edu.cn

advertisement in communities. They were required to be Guangzhou residents for at least 1 year, and be free of the following conditions: confirmed prevalent diseases of the digestive system, use of hormone or antibiotics within 30 d before the study and pregnancy. Potential candidates were then invited to the First or the Second Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Investigators verified the subjects' eligibility via face-to-face interview. We recruited equal number of twenty-one subjects in each sex–age subgroups (20–29, 30–39, 40–49, 50–59 and 60–69 years). A total of 210 subjects were finally enrolled.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all protocols involving human subjects were approved by the Medical Ethics Committee of Sun Yat-sen University and all participants signed the written informed consent.

Information on sociodemographic data, medication and health history, lifestyles, physical condition, and female menstrual and menopausal status were collected by trained interviewers at face-to-face interviews using a structured questionnaire. A 3 d 24 h food diary (2 d before urinary collection and 1 d during the period of urine collection) was completed by each subject and checked by researchers. Each subject collected a pooled 24 h urine sample while on his/her usual diet. Urine volumes were recorded, and 10 ml samples were stored at −80°C before analysis. Participants were asked to maintain their regular diet, but avoid alcohol consumption during the 3 d food diary collection. After the completion of the first urine collection, participants were given two capsules of isoflavone extracts for 3 d. Each capsule contained 24·06 mg daidzein, 5·82 mg genistein, 11·87 mg glycitein and in total 40 mg soya isoflavones (all in aglycone equivalent). Participants were requested to record their physical condition and report any adverse events, a 3 d food diary and any medications used. A 24 h pooled urine sample was collected on the third day of the soya isoflavone challenge. Participants were asked to avoid soya and soya products during the soya isoflavone challenge test to avoid ingestion of additional soya isoflavones. Alcohol was also prohibited during the period of the soya isoflavone challenge test.

Eight subjects (three men and three pre- and two postmenopausal women) were excluded from data analysis for reasons of failure to complete the urine collection or food diary, or because of poor compliance with the isoflavone supplement. A total of 202 participants successfully completed the study, and were included in the data analysis.

Measurements

On the days of urine collection before or after an isoflavone load, height, weight, waist and hip circumferences and blood pressure were measured. For biochemical analysis, 12 h fasting venous blood was collected in vacuum tubes containing EDTA. Plasma was separated after centrifugation at 1500 g for 15 min at 4°C within 2 h and was stored at −80°C till tests. Blood lipids, glucose and uric acid were measured using Hitachi 7600-010 automatic analyser. The CV for lipid measurements were 2·17 % (at 5·03 mmol/l total cholesterol), 2·86 % (at 1·14 mmol/l TAG), 3·47 % (at 1·70 mmol/l HDL-cholesterol), 4·67 % (at 2·65 mmol/l LDL-cholesterol) and 2·15 % (at 290·2 μmol/l uric acid). Biochemical assays were performed by two technicians who were not involved in the questionnaire interview.

Equol, *O*-DMA, daidzein, genistein and glycitein and total isoflavones in 24 h urine were assayed by HPLC with UV detection[17]. Urine samples were extracted by ethyl acetate (5 ml × 2) after deconjugation by β-glucuronidase/sulphatase. After reduced pressure drying, the extract was reconstituted in mobile phase solution (1 ml) for analysis. The HPLC system consisted of a C_{18} stationary phase extraction (5 μm, 4·6ø × 250 mm) column, and separation of isoflavones was achieved by gradient elution with the mobile phase of 20–70 % methanol at a flow rate of 1·0 ml/min. Isoflavones were detected from their UV absorbance at 254 nm and 280 nm. The CV for daidzein, equol and *O*-DMA measurements were <5 %.

Subjects with 24 h urinary equol:daidzein and *O*-DMA:daidzein ratios >0·018 were defined as equol and *O*-DMA excretors on their usual diet and producers after a soya isoflavone challenge[18]. All excretors at baseline were identified as producers.

Data analysis

Data were checked for normality, and skewed parameters were log transformed before statistical analysis when possible. Data that were normally distributed either before or after log transformation were presented as means and standard deviations. For TAG, we used winsorisation to replace outliers that were at least three SD away from the group mean with the next most extreme value (four samples), as the few outliers affected the mean values extremely.

Comparisons of variables between the different daidzein-metabolising phenotypes were done using either Student's *t* test or χ^2 test. All analyses were conducted with SPSS for Windows (version 11.5; SPSS, Inc., Chicago, IL, USA).

Results

On their usual diet, 27 ((SE 13·4) %) and 27 ((SE 13·4) %) of 202 subjects had detectable urinary equol or *O*-DMA excretion, respectively, while 9 ((SE 4·5) %) excreted both equol and *O*-DMA. After a soya isoflavone challenge, 50 % (*n* 101), 46·5 % (*n* 94) and 25·7 % (*n* 52) of the subjects were classified as producers of equol, *O*-DMA and both, respectively. The ratios of equol:daidzein and *O*-DMA:daidzein ranged between 0·14–4·53 and 0·08–2·12 in the excretors, and between 0·03–5·83 and 0·03–1·47 in the producers, respectively. The proportions of equol or *O*-DMA producers were 55 and 51 % in women, and 45 and 42 % in men, respectively. There was no statistical difference in the proportion of equol and/or *O*-DMA excretors/producers among sex and age groups ($P>0·05$; data not shown). After 80 mg/d isoflavone load, 101, 94, 199, 198 and 198 of 202 subjects had detectable equol, *O*-DMA, daidzein, genistein and glycitein in 24 h urine, respectively. Table 1 shows the mean concentrations. Among those with detected urinary isoflavones or metabolites, mean values were 14·31 (SD 14·41) (equol), 6·16 (SD 6·67) (*O*-DMA), 30·32 (SD 24·08) (daidzein), 3·13 (SD 2·56) (genistein) and 11·32 (SD 7·44) (glycitein) μmol/l, respectively.

Table 1. Twenty-four-hour urinary isoflavonoid metabolic production in each age group by sex (μmol/l)

(Mean values and standard deviations)

	Total			20–29 years			30–39 years			40–49 years			50–59 years			60–69 years		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Total subjects	202			42			39			40			41			40		
Equol producer	101	14·31	14·41	18	15·22	13·13	24	10·75	10·10	19	15·26	11·09	17	16·17	21·94	23	15·13	15·30
O-DMA producer	94	6·16	6·67	24	5·99	3·98	11	3·96	2·01	19	9·56	11·26	15	6·02	7·06	25	4·78	4·21
Daidzein producer	199	30·32	24·08	42	36·38	25·89	38	29·26	16·82	39	32·37	27·98	41	27·69	30·94	39	25·62	12·95
Genistein producer	198	3·13	2·56	42	4·11	3·05	38	3·50	2·59	38	3·26	2·80	41	2·42	1·52	39	2·35	2·23
Glyscitein producer	198	11·32	7·44	42	14·37	10·79	38	10·97	6·56	38	11·69	7·35	41	9·16	4·48	39	10·34	5·49
Male subjects	102			21			20			20			20			21		
Equol producer	46	13·72	11·48	7	17·29	17·96	10	10·20	12·54	9	15·67	8·40	8	13·25	11·82	12	13·46	8·76
O-DMA producer	44	5·84	7·59	9	5·81	2·15	5	3·17	1·01	9	11·20	15·63	8	4·60	3·26	13	3·94	2·09
Daidzein producer	99	30·30	20·90	21	33·60	15·61	19	33·29	20·48	18	37·95	34·76	20	24·09	12·17	21	22·77	9·96
Genistein producer	99	3·27	2·43	21	3·94	2·44	19	4·39	3·21	18	3·22	2·46	20	2·85	1·70	21	2·00	1·44
Glyscitein producer	98	11·18	8·05	21	14·29	7·02	19	11·91	8·30	17	13·19	7·48	20	9·05	5·37	21	8·94	4·06
Female subjects	100			21			19			20			21			19		
Equol producer	55	14·82	16·6	11	13·91	9·73	14	11·14	8·43	10	14·90	13·53	9	18·78	28·70	11	17·09	20·93
O-DMA producer	50	6·44	5·80	15	6·10	4·84	6	4·61	2·48	10	8·07	5·59	7	7·65	9·90	12	5·69	5·67
Daidzein producer	100	30·33	27·04	21	39·16	33·38	19	25·02	10·84	20	26·79	18·26	21	31·12	41·81	19	28·77	15·27
Genistein producer	99	2·99	2·69	21	4·28	3·61	19	2·55	1·23	20	3·32	2·46	21	2·01	1·24	18	2·74	2·85
Glyscitein producer	100	11·46	6·82	21	14·45	13·76	19	9·97	4·03	20	10·26	7·12	21	9·26	3·56	19	11·89	6·49

O-DMA, *O*-desmethylangolensin.

Of 202 volunteers, 27 % consumed soya or soya products on the day or the day before 24 h urine sampling during the usual diet period. The proportion of equol and *O*-DMA excretors was significantly higher in soya food consumers (26 and 24 %, respectively) than in non-consumers (10 and 11 %, respectively; $P=0{\cdot}008$ and 0·014, respectively).

There was no significant association between equol-metabolising phenotype and demographic factors of subjects, such as age, and economic, education and marital status (Table 2). There was a significant difference in the proportion of *O*-DMA-metabolising phenotypes among different education levels. On isoflavone challenge days, subjects with different daidzein-metabolising phenotypes had no significant differences in dietary intakes of cereals and grains, poultry, fish and shellfish, red meat, vegetables, fruits and eggs. However, *O*-DMA producers consumed significantly higher amounts of dairy products than non-producers.

Equol producers showed lower serum uric acid (−10·2 %, $P=0{\cdot}001$), TAG (−29·5 %, $P=0{\cdot}007$) and waist:hip ratio (−2·6 %, $P=0{\cdot}032$), and tended to have higher HDL-cholesterol (6·3 %, $P=0{\cdot}069$) compared with the non-producers in total subjects. The differences in uric acid, waist:hip ratio, TAG and HDL-cholesterol between equol producers and non-producers were more pronounced in men than in women. On the other hand, women equol producers showed significantly lower LDL-cholesterol than non-producers ($P=0{\cdot}040$). No significant differences in fasting blood glucose or blood pressure were observed between equol producers and non-producers, whether in the overall group or in men and women separately. There were no significant differences in serum lipids, glucose, blood pressure and uric acid between *O*-DMA producers and non-producers (Table 3).

Discussion

Equol producers in the present study represented 50 % of the subjects. The present finding is consistent with the previously reported frequency of equol producers in Asians[12,13]. While the prevalence of *O*-DMA producers in the present study was 46·5 %, this figure is lower than that reported in some studies (80–90 % generally)[10,11]. The present study subjects were more likely to be equol producers and less likely to be *O*-DMA producers compared with the western populations[10]. It has been suggested that racial differences and genetic predisposition may influence the ability to metabolise daidzein to *O*-DMA[13]. The proportion of *O*-DMA producers in the present study is also lower than that in Japan[12]. Some studies reported that intestinal microbial populations had geographic differences[19], suggesting that the *O*-DMA-metabolising difference in the Japanese might be due to differences in the environment.

A prior study reported the effect of age and sex on the bioavailability of isoflavones in healthy adults, but no age or sex differences in equol production were observed in that study[20]. Some studies suggested that age might influence the ability to produce equol because of a cohort effect or age-related changes in intestinal bacterial populations[21,22]. In two cross-sectional studies, bacterial diversity in healthy young adults was greater than the diversity in healthy elderly adults[21,22], suggesting age-related changes in intestinal

Table 2. Participant characteristics by equol-producer and *O*-desmethylangolensin (*O*-DMA)-producer status after a 3-d isoflavone challenge (Mean values and standard deviations or percentages)

	Equol					*O*-DMA				
	Producers		Non-producers			Producers		Non-producers		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
Demographics										
Age (years)*	44·3	13·9	43·5	14·3	0·688	44·1	15·4	43·6	12·9	0·797
Education (%)†					0·221					0·012
Primary/middle school	33·0		22·2			28·6		25·8		
High school	29·5		38·9			23·8		44·1		
College and above	37·5		38·9			47·6		30·1		
Monthly income per capita (¥, %)†					0·404					0·771
<1000	33·0		37·8			32·1		38·7		
1000–2000	33·0		37·8			26·9		34·4		
>2000	34·1		24·4			31·0		26·9		
Marital status (%)†					0·172					0·423
Married	76·1		66·7			69·0		73·1		
Divorced/widowed	2·3		7·8			3·6		6·5		
Single	21·6		25·6			27·4		20·4		
Dietary measures (servings/d)*										
Cereals and grains	3·6	0·9	3·4	0·9	0·253	3·6	0·9	3·4	0·8	0·364
Poultry	0·6	0·5	0·7	0·6	0·153	0·6	0·5	0·6	0·6	0·693
Fish and shellfish	0·7	0·6	0·8	0·6	0·246	0·8	0·6	0·7	0·6	0·337
Red meat	1·5	0·7	1·6	0·7	0·563	1·5	0·7	1·6	0·7	0·376
Vegetables	2·6	1·0	2·7	1·0	0·537	2·7	1·0	2·7	0·9	0·693
Fruits	0·7	0·7	0·6	0·6	0·246	0·7	0·8	0·6	0·6	0·217
Dairy foods	0·2	0·4	0·2	0·5	0·953	0·3	0·5	0·2	0·3	0·043
Eggs	0·5	0·5	0·5	0·5	0·843	0·5	0·5	0·5	0·5	0·599

* Student's *t* test.
† χ^2 test.

Table 3. Serum glucose, lipids and uric acid in southern Chinese adults by producers of daidzein-metabolising phenotypes*
(Mean values and standard deviations)

	Equol					*O*-DMA				
	Producers		Non-producers			Producers		Non-producers		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
Total										
BMI (kg/m^2)	22·4	3·1	22·6	3·3	0·685	22·4	3·2	22·7	3·3	0·550
Waist:hip ratio	0·839	0·069	0·861	0·076	0·032	0·848	0·076	0·851	0·071	0·833
SBP (mmHg)	114·7	18·2	115·0	18·3	0·914	113·3	19·3	116·6	16·9	0·197
DBP (mmHg)	78·4	14·7	81·5	20·0	0·221	78·2	19·1	80·8	14·6	0·277
Glucose (mmol/l)	5·08	0·96	5·22	1·38	0·391	5·15	1·10	5·15	1·27	0·964
TC (mmol/l)	5·55	1·10	5·76	1·09	0·160	5·68	1·08	5·61	1·09	0·663
TAG (mmol/l)†	1·46	1·31	2·07	2·09	0·007	1·63	1·42	1·88	2·02	0·557
LDLc (mmol/l)	3·33	0·88	3·45	0·87	0·332	3·45	0·87	3·32	0·84	0·256
HDLc (mmol/l)	1·58	0·42	1·48	0·36	0·069	1·53	0·42	1·53	0·37	0·995
Uric acid (μmol/l)	312·1	65·9	347·6	75·0	0·001	322·9	69·5	334·4	74·4	0·263
Female										
BMI (kg/m^2)	22·5	3·1	22·0	3·2	0·447	22·0	3·3	22·5	3·0	0·359
Waist:hip ratio	0·813	0·058	0·824	0·072	0·393	0·820	0·072	0·816	0·057	0·752
SBP (mmHg)	113·0	18·7	109·6	18·5	0·359	108·6	18·3	114·3	18·6	0·128
DBP (mmHg)	76·1	16·3	80·5	25·3	0·295	76·9	23·0	79·4	18·6	0·563
Glucose (mmol/l)	4·86	0·53	5·09	0·95	0·129	4·91	0·79	5·01	0·72	0·511
TC (mmol/l)	5·60	1·03	5·95	0·98	0·081	5·79	0·92	5·72	1·11	0·729
TAG (mmol/l)†	1·26	0·64	1·40	0·80	0·527	1·36	0·76	1·28	0·68	0·694
LDLc (mmol/l)	3·29	0·85	3·64	0·84	0·040	3·44	0·78	3·45	0·93	0·962
HDLc (mmol/l)	1·71	0·45	1·68	0·30	0·635	1·72	0·45	1·68	0·31	0·586
Uric acid (μmol/l)	284·0	61·2	312·1	68·7	0·034	294·5	68·4	298·5	63·7	0·763
Male										
BMI (kg/m^2)	22·4	3·2	23·1	3·4	0·260	22·9	3·1	22·8	3·5	0·861
Waist:hip ratio	0·869	0·068	0·892	0·066	0·099	0·881	0·067	0·880	0·068	0·984
SBP (mmHg)	116·8	17·4	119·5	17·0	0·429	118·8	19·2	118·5	15·1	0·928
DBP (mmHg)	81·2	12·1	82·3	14·4	0·693	79·7	13·2	82·0	9·9	0·322
Glucose (mmol/l)	5·34	1·26	5·33	1·65	0·968	5·41	1·33	5·27	1·59	0·638
TC (mmol/l)	5·49	1·18	5·61	1·17	0·594	5·55	1·24	5·52	1·07	0·898
TAG (mmol/l)†	1·71	1·80	2·61	2·60	0·013	1·95	1·87	2·40	2·59	0·498
LDLc (mmol/l)	3·39	0·91	3·30	0·88	0·633	3·46	0·97	3·20	0·73	0·120
HDLc (mmol/l)	1·41	0·32	1·32	0·32	0·127	1·30	0·23	1·40	0·37	0·141
Uric acid (μmol/l)	345·5	55·5	375·4	68·1	0·019	355·2	55·8	365·3	69·2	0·435

O-DMA, *O*-desmethylangolensin; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDLc, HDL-cholesterol; LDLc, LDL-cholesterol.
* Student's *t* test
† Log transformation.

microflora, but this hypothesis has not yet been evaluated prospectively. Also, it was suggested that there were age differences in the bacteria responsible for the equol-producing phenotypes[21]. In the present study, women tended to have a higher proportion of equol and *O*-DMA producers than men, but this sex difference was not statistically significant, possibly due to the small study size. In contrast, Setchell & Cole[18] observed that the propensity to make equol was higher in males than in females. Further larger studies are needed to clarify the sex and/or age differences in equol/*O*-DMA producers.

Concurring with the findings in supplementary studies[8–9], the present study showed that equol production was positively associated with soya intake. We found a significantly higher excretion of isoflavones in Chinese adults who had consumed soya on the last day before the urine collections than in those who did not. Thus, the lower amounts of equol reported in cross-sectional studies might only reflect recent soya intake. The intake of soya products might thus explain why there was a lower proportion of subjects excreting equol/*O*-DMA on their usual diet than of those producing these metabolites after an isoflavone load.

The aim of the present study was also to investigate the relationships between the equol or *O*-DMA phenotypes and some cardiovascular risk factors. A recent advisory by the American Heart Association Nutrition Committee concluded that the beneficial effects of soya or isoflavones supplementation on cardiovascular health were minimal at best, with recent studies revealing no significant improvement in blood pressure or the lipid profile[23]. However, prior studies observed that equol production was associated with a lower risk of CVD[24]. The limited cardiovascular benefits of isoflavones supplementation in human subjects were postulated to be due to inter-individual variation in the ability to produce equol. Consistent with this hypothesis, we observed a favourable relationship between blood lipids and the equol phenotype among Chinese adults, suggesting that the ability to produce equol might play a role in modulating cardiovascular risk factors. It has also been reported that serum uric acid levels are associated with the risk of CVD[25]. From our knowledge, the present study was the first study that found a lower level of serum uric acid in the equol producers than in non-producers in Chinese adults.

In the present study, we found that the favourable differences in uric acid, waist:hip ratio, TAG and HDL-cholesterol between equol producers and non-producers were more pronounced in men than in women. Similarly, we also observed more beneficial effects of soya on serum lipids in men than in women in our previous cross-sectional study[26]. The reasons for such sex differences remain unclear. As discussed in the previous study[26], menopause might attenuate the association between equol production and cardiovascular risks in our female subjects, since about a half of them were pre- or post-menopausal.

In order to avoid misclassifying equol/*O*-DMA producers, we defined them by the equol or *O*-DMA:daidzein ratio, since this is a product–precursor relationship. After we detected equol and *O*-DMA excretors on their usual diet, we used an isoflavone challenge to determine producers. The frequency of equol and *O*-DMA excretors increased after a soya isoflavone challenge. The possibility exists that many of our subjects had the ability to produce equol or *O*-DMA but did not because of limited soya intake. Consequently, some equol or *O*-DMA producers might not produce any equol or *O*-DMA due to limited or no soya food in their usual diets. Moreover, we found that the productions of equal and *O*-DMA were highly variable among individuals following the same dose of isoflavones.

In some studies[9–11], given the widespread ability to produce *O*-DMA, the assessment of differences between *O*-DMA producers and non-producers was limited due to the relatively small percentage of *O*-DMA non-producers. In the present study, 46·5 % of subjects were *O*-DMA producers. However, we did not observe any differences in blood lipids and uric acid between *O*-DMA producers and non-producers. It has been suggested that *O*-DMA production has no effect on the prevention of CVD[27].

The major limitation of the present study was that we did not have enough power to detect small differences in blood lipids due to the relatively small study size. The results of the present study should be confirmed in a larger, more diverse sample. Moreover, high variability in the analytical methods of equol and *O*-DMA might also attenuate the associations between equol or *O*-DMA phenotypes and blood lipids. In addition, we excluded only participants who had taken antibiotics within 1 month before the study. Antibiotic treatment for up to 3 months might cause changes in intestinal bacterial population and decrease the prevalence of equol producers. In this case, we might have underestimated the prevalence of equol producers, and some producers could have been misclassified. However, these limitations were unlikely to lead to the overestimation of the equol–lipids associations observed in the present study.

Overall, the present findings suggest that equol phenotypes might influence cardiovascular risk, and further studies are warranted to show whether daidzein-metabolising phenotypes determine the efficacy of soya isoflavones in preventing CVD.

Acknowledgements

The present study was supported by Otsuka Pharmaceutical Company, Limited, Japan. The authors' responsibilities were as follows: K. P. G., B. Z., Y. M. C. and C. G. C. contributed to the data collection, analysis and the manuscript preparation. S. U. and T. U. were responsible for the urinary analysis. Y. X. S. was responsible for the conception and design of the study, and Y. X. S. and Y. M. C. were responsible for the critical revision of the manuscript. S. U. and T. U. were the employees of Otsuka Pharmaceutical Company. None of the other authors have any other financial or personal conflict of interest. The authors wish to thank the volunteers who have entered the study. They are grateful to Dr Setchell kindly reviewing the manuscript. They specially thank Steve Bannister for his kind help with the English language.

References

1. Setchell KD (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* **68**, 1333S–1346S.
2. Hwang CS, Kwak HS, Lim HJ, *et al.* (2006) Isoflavone metabolites and their *in vitro* dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *J Steroid Biochem Mol Biol* **101**, 246–253.
3. Lichtenstein AH (1998) Soy protein, isoflavones and cardiovascular disease risk. *J Nutr* **128**, 1589–1592.
4. Nagata C (2000) Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan. *Int J Epidemiol* **29**, 832–836.
5. Setchell KD, Clerici C, Lephart ED, *et al.* (2005) *S*-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* **81**, 1072–1109.
6. Schmitt E, Dekant W & Stopper H (2001) Assaying the estrogenicity of phytoestrogens in cells of different estrogen sensitive tissues. *Toxicol In Vitro* **15**, 433–439.
7. Lampe JW, Skor HE, Li S, *et al.* (2001) Wheat bran and soy protein feeding do not alter urinary excretion of the isoflavan equol in premenopausal women. *J Nutr* **131**, 740–744.
8. Rowland IR, Wiseman H, Sanders TA, *et al.* (2000) Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. *Nutr Cancer* **36**, 27–32.
9. Duncan AM, Merz-Demlow BE, Xu X, *et al.* (2000) Premenopausal equol excretors show plasma hormone profiles associated with lowered risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* **9**, 581–586.
10. Atkinson C, Newton KM, Bowles EJ, *et al.* (2008) Demographic, anthropometric, and lifestyle factors and dietary intakes in relation to daidzein-metabolizing phenotypes among premenopausal women in the United States. *Am J Clin Nutr* **87**, 679–687.
11. Song KB, Atkinson C, Frankenfeld CL, *et al.* (2006) Prevalence of daidzein-metabolizing phenotypes differs between Caucasian and Korean American women and girls. *J Nutr* **136**, 1347–1351.
12. Watanabe S, Yamaguchi M, Sobue T, *et al.* (1998) Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). *J Nutr* **128**, 1710–1715.
13. Arai Y, Uehara M, Sato Y, *et al.* (2000) Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. *J Epidemiol* **10**, 127–135.
14. Setchell KD, Brown NM & Lydeking-Olsen E (2002) The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. *J Nutr* **132**, 3577–3584.
15. Frankenfeld CL, McTiernan A, Aiello EJ, *et al.* (2004) Mammographic density in relation to daidzein-metabolizing phenotypes in overweight, postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **13**, 1156–1162.

16. Frankenfeld CL, McTiernan A, Thomas WK, *et al.* (2006) Postmenopausal bone mineral density in relation to soy isoflavone-metabolizing phenotypes. *Maturitas* **53**, 315–324.
17. Lundh TJ, Pettersson H & Kiessling KH (1988) Liquid chromatographic determination of the estrogens daidzein, formononetin, coumestrol, and equol in bovine blood plasma and urine. *J Assoc Off Anal Chem* **71**, 938–941.
18. Setchell KDR & Cole SJ (2006) Method of defining equol-producer status and its frequency among vegetarians. *J Nutr* **136**, 2188–2193.
19. Moore WE & Moore LH (1995) Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol* **61**, 3202–3207.
20. Cassidy A, Brown JE, Hawdon A, *et al.* (2006) Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J Nutr* **136**, 45–51.
21. Hopkins MJ & Macfarlane GT (2002) Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *J Med Microbiol* **51**, 448–454.
22. Hopkins MJ, Sharp R & Macfarlane GT (2001) Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* **48**, 198–205.
23. Sacks FM, Lichtenstein A, Van Horn L, *et al.* (2006) Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. *Circulation* **113**, 1034–1044.
24. Kreijkamp-Kaspers S, Kok L, Bots ML, *et al.* (2005) Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women. *Am J Clin Nutr* **81**, 189–195.
25. Fang J & Alderman MH (2000) Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971–1992. National Health and Nutrition Examination Survey. *JAMA* **283**, 2404–2410.
26. Zhang B, Chen YM, Huang LL, *et al.* (2008) Greater habitual soyfood consumption is associated with decreased carotid intima-media thickness and better plasma lipids in Chinese middle-aged adults. *Atherosclerosis* **198**, 403–411.
27. Howes JB, Sullivan D, Lai N, *et al.* (2000) The effects of dietary supplementation with isoflavones from red clover on the lipoprotein profiles of post menopausal women with mild to moderate hypercholesterolaemia. *Atherosclerosis* **152**, 143–147.

British Journal of Nutrition