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Urinary isoflavonoids and risk of type 2 diabetes: a prospective investigation in US women

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Abstract

To examine the association between urinary excretion of isoflavonoids and risk of type 2 diabetes (T2D), we conducted a nested case–control study among 1111 T2D pairs identified during 1995–2008 in the Nurses' Health Study (NHS) and NHSII, who were free of diabetes, CVD and cancer at urine sample collection. Urinary excretion of daidzein and genistein, as well as their metabolites *O*-desmethylangolensin (*O*-DMA), dihydrogenistein (DHGE) and dihydrodaidzein (DHDE) was assayed using liquid chromatography MS. Self-reported T2D incident cases were confirmed using a validated questionnaire. Higher urinary excretion of daidzein and genistein was associated with a lower risk of T2D in the combined cohorts. Comparing extreme tertiles of the urinary markers, the OR of T2D were 0·71 (95 % CI 0·55, 0·93) for daidzein and 0·74 (95 % CI 0·56, 0·97) for genistein, although the test for linear trend was not significant for genistein (P_{trend} = 0·03 and 0·15, respectively). DMA, DHDE and DHGE were non-significantly associated with a lower T2D risk. The inverse association of daidzein with T2D risk was stronger among post-menopausal women who did not use hormone replacement therapy ($P_{interaction}$ = 0·001): the OR was 0·58 (95 % CI 0·34, 0·97) comparing extreme tertiles among these women. In conclusion, urinary excretion of isoflavones was associated with a lower T2D risk in US women, especially among post-menopausal women who did not use hormone. Further research is warranted to replicate these observations among western populations with similarly low overall isoflavone intake.

Key words: Isoflavones: Diabetes: Menopausal status: Nested case–control studies

Type 2 diabetes (T2D) is a chronic disease with an increasing prevalence worldwide. The total number of diabetes patients is estimated to reach 592 million globally by the year 2035[1]. Excessive body weight, unhealthy diet, lack of exercise and smoking are major risk factors of T2D[2]. Seeking effective dietary and lifestyle measures for T2D prevention has been a priority to counteract the increasing prevalence and incidence of diabetes[3–5]. Women may be at a particularly high risk at middle life when menopause and ageing jointly increase the risk of T2D[6–8]. Large clinical trials and prospective cohort studies have consistently shown that hormone replacement therapy may reduce the risk of T2D in post-menopausal women[9–12]. However, it is unclear whether natural phytoestrogens, such as isoflavones, may be associated with T2D risk.

Clinical trials showed that isoflavone supplements did not improve glucose control[13,14], although these clinical trials were limited by small sample size and short duration of follow-up. Several cohort studies have evaluated the association of isoflavone intake assessed using FFQ with risk of T2D, and mixed results were observed[15–17]. One potential reason for the inconsistent findings in the observational studies may lie in the difficulties of using FFQ to assess isoflavone intakes. Soya foods are the main source of isoflavone intakes, although other foods contain various amounts of isoflavones as well[18]. Isoflavone intake estimated from FFQ is thus subject to measurement errors[19], especially in Western populations who infrequently consume soya foods. In addition, isoflavone intake estimated from FFQ does not take into account inter-individual variations in bioavailability[20]. In addition, FFQ cannot be used to estimate the gut microbiota metabolites of isoflavones – including *O*-desmethylangolensin (*O*-DMA)[21], dihydrogenistein (DHGE) and dihydrodaidzein (DHDE) – and equol[22], which may exert

Abbreviations: DHDE, dihydrodaidzein; DHGE, dihydrogenistein; *O*-DMA, *O*-desmethylangolensin; NHS, Nurses' Health Study; T2D, type 2 diabetes.

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biological effects in addition to their parent compounds (i.e. daidzein and genistein)[23]. In this regard, use of isoflavone metabolites in blood or urine as objective markers of isoflavone intake is an appealing approach[24,25].

In the current investigation, we utilised data from a combined cohort based on two well-characterised cohorts of US women, the Nurses' Health Study (NHS) and NHSII, to prospectively evaluate the association of urinary excretion of isoflavone metabolites with risk of T2D. We also examined the hypothesis that isoflavone excretion may especially be associated with lower T2D risk among post-menopausal women who do not receive replacement therapy and thus have a low exposure to exogenous estrogens.

Methods

Study population

The NHS began in 1976, when 121 700 female registered nurses aged 30–55 years residing in eleven states were enroled and completed a baseline questionnaire about their lifestyle and medical history. The NHSII was established in 1989 and consisted of 116 430 younger female registered nurses aged 25–42 years at baseline. These nurses also responded to a baseline questionnaire similar to that of the NHS. In both cohorts, questionnaires were collected at baseline and biennially thereafter, to update information on age, weight, smoking status, physical activity, medication use, menopausal status, post-menopausal hormone use and disease status – including hypertension, hypercholesterolaemia, CVD and cancer.

Urine sample collection

A total of 18 743 NHS participants aged 53 to 80 years provided morning spot urine samples from 2000 to 2002, and 29 611 NHSII participants aged 32 to 52 years provided morning spot urine samples from 1996 to 1999. For both cohorts, the samples were returned to a central biorepository Via overnight courier and were immediately processed upon arrival and aliquoted into cryotubes, which were stored in the vapour phase of liquid nitrogen freezers at ≤ -130 °C. Loss to follow-up was <10 % among participants who provided blood and urine samples.

Prospective case–control study design

We conducted a prospective, nested case–control study among participants who provided urine samples and were free of self-reported diabetes, CVD and cancer at urine collection in NHS and NHSII. T2D cases diagnosed within the 1st year since urine sample collection were excluded from selection in order to reduce the potential for reverse causation bias. During follow-up from urine collection through 2008 (NHS)/2007 (NHSII), we prospectively identified and confirmed 1111 T2D cases (NHS: 456; NHSII: 655) and randomly selected one control from diabetes-free participants using risk set sampling[26] The cases and controls were matched for age at sample collection, month of sample collection, fasting status (≥8 h or not), first morning urine (yes or no) and race (White or other races) in both cohorts. In NHSII, we additionally matched for menopausal status (yes, no), luteal day of the menstrual cycle (date of next period minus date of sample collection) for pre-menopausal women and hormone replacement therapy (yes or no) for post-menopausal women. The study protocol was approved by the institutional review board of Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

Ascertainment of type 2 diabetes

We sent a validated supplemental questionnaire to those who reported a physician diagnosis of T2D to confirm the incidence[27]. We used at least one of the following American Diabetes Association 1998 criteria to confirm self-reported T2D diagnosis: an elevated glucose concentration (fasting plasma glucose ≥7·0 mmol/l, random plasma glucose ≥11·1 mmol/l, or plasma glucose ≥11·1 mmol/l after an oral glucose load) and at least one symptom related to diabetes; no symptoms but elevated glucose concentrations on two separate occasions; or treatment with insulin or oral hypoglycaemic medication. Only confirmed T2D cases were included in the current study.

Assessment of diet

Validated FFQ have been administered since 1984 in NHS and since 1991 in NHSII[28]. Similar FFQ were subsequently sent to participants every 2–4 years to update diet. In these FFQ, we enquired about the consumption frequency of 118–166 food items in the past year, and how often (from 'never or less than once per month' to 'six or more times per day') on an average they consumed each food item of a standard portion size. Major soya foods, such as tofu and soya milk, have been simultaneously included on the FFQ since 1998 in the NHS and since 1999 in the NHSII. An overall measure of diet quality was calculated using the Alternate Healthy Eating Index (AHEI) score, excluding the soya food items, such as tofu and soya milk[29]. AHEI is based on foods and nutrients that are predictive of the risk of developing major chronic diseases. To calculate AHEI score, we assigned individual score to each of the food group and summed up the scores, with a higher score indicative of better diet quality[30].

Laboratory measurements

In the current study, we used electrospray ionisation liquid chromatography MS to measure isoflavonoids in urine samples, which has been validated except for the use of an orbitrap mass spectrometer[21,31]. Urinary creatinine levels were measured using a Roche-Cobas MiraPlus clinical chemistry autoanalyzer (Roche Diagnostics). The average intra-assay CV was 4·1 % for daidzein, 7·6 % for genistein, 8·2 % for DHDE, 10·1 % for DHGE, 8·1 % for *O*-DMA and 5·6 % for creatinine. We calculated creatinine-adjusted concentrations (nmol/g creatinine) of isoflavonoids by dividing the isoflavonoid levels (nmol/l) by creatinine levels (g/l). In a pilot study that evaluated within-person stability of the isoflavonoids, intra-class correlation coefficients (ICC) of two urine samples from 58 NHSII participants

collected 1–2 years apart were 0·05 for daidzein, 0·14 for genistein, 0·16 for DHDE, 0·14 for DHGE and 0·20 for *O*-DMA.

Statistical methods

We calculated Spearman correlation coefficients between urine excretions of isoflavone metabolites with soya foods, such as tofu and soya milk, estimated from FFQ. We adjusted for total energy intake (kJ/d (kcal/d)), BMI (kg/m^2), physical activity (MET-h/week), age (years), smoking (never, past, current) and first morning urine (yes or no). This analysis was conducted among controls to facilitate comparison between cohorts.

We categorised the isoflavone biomarkers into tertiles. We used conditional logistic regression stratified by matching factors to model the association between isoflavone metabolites and risk of T2D in the main analysis[26]. We additionally adjusted for hypertension at baseline (yes or no), hypercholesterolaemia at baseline (yes or no), BMI (kg/m^2), smoking (non-smoker, past smoker, current smoker), AHEI score, physical activity (MET-h/week), total energy intake (kJ/d (kcal/d)), menopausal status (pre-menopausal or post-menopausal) (NHS only) and hormone replacement therapy (yes or no) (NHS only). *P* values for linear trend were calculated by examining an ordinal score based on the median value in each tertile of isoflavones biomarker levels in the multivariate models.

Given that menopausal status and post-menopausal hormone therapy were not matching factors in NHS, we conducted stratified analyses by menopausal status and post-menopausal hormone therapy (yes *v.* no) using unconditional logistic regression to maximise statistical power. *P* values for interactions were evaluated using the likelihood ratio test, comparing the multivariate model with and without interaction terms of dichotomised isoflavones and potential effect modifiers in conditional logistic regression. Joint associations of the urinary biomarkers and potential effect modifiers were estimated using conditional logistic regression. All *P* values were two-sided. Data were analysed with the statistical analysis systems software package, version 9.3 (SAS Institute Inc.).

Results

Baseline characteristics of study participants are shown in Table 1. Compared with controls, T2D cases consumed a less healthful diet, engaged in less physical activity, had a higher BMI and were more likely to have a history of hypertension and hypercholesterolaemia. The mean value of the urine isoflavones was 3863 nmol/g creatinine among cases and 5435 nmol/g creatinine among controls. The baseline characteristics according to urine isoflavone excretion are shown in online Supplementary Table S1. Higher urinary isoflavone excretion was correlated with a healthier dietary pattern and higher levels of physical activity. We further compared the baseline characteristics of controls with that of the population, and the controls were comparable to the total participants in NHS and NHSII (online Supplementary Table S2).

Moderate to strong correlations among urinary isoflavone metabolites were observed (correlation coefficient: 0·26–0·79), with the strongest correlation observed between daidzein and genistein (online Supplementary Table S3). Weak yet significant correlations were found between soya foods estimated from FFQ and urinary isoflavone excretion among controls.

Urinary excretion of total isoflavones was not associated with risk of T2D (P_{trend} = 0·20). Higher levels of daidzein and genistein, which are the dominant metabolites of isoflavones, were associated with a lower risk of T2D (Table 2). For daidzein, compared with the lowest group, the OR of T2D were 0·78 (95 % CI 0·60, 1·02) in the second tertile and 0·71 (95 % CI 0·55, 0·93) in the highest tertile (P_{trend} = 0·03); for genistein, compared with the lowest group, the OR of T2D were 0·70 (95 % CI 0·53, 0·93) in the second tertile and 0·74 (95 % CI 0·56, 0·97) in the highest tertile (P_{trend} = 0·15). *O*-DMA, DHDE and DHGE – which are the metabolites of daidzein and genistein – were non-significantly associated with a lower risk of T2D. Comparing the extreme tertiles, the OR of T2D were 0·92 (95 % CI 0·70, 1·21) for *O*-DMA, 0·80 (95 % CI 0·60, 1·06) for DHDE and 0·82 (95 % 0·62, 1·08) for DHGE. Further adjustment of individual dietary components – such as red meats, fruits and vegetables, and coffee – in replacement of AHEI did not change the results materially. In a sensitivity analysis, we further excluded 945 participants whose HbA_{1c} levels were ≥5·7 % and repeated the analysis. The inverse association between daidzein and risk of T2D persisted: compared with the lowest tertile, the OR of T2D were 0·91 (95 % CI 0·47, 1·77) in the second tertile and 0·49 (95 % CI 0·26, 0·94) in the highest tertile (P_{trend} = 0·01).

Stratified analyses by menopausal status and hormone use were conducted. Significant interaction by post-menopausal hormone use was found for daidzein ($P_{interaction}$ = 0·001) (Table 3). Comparing extreme tertiles of the urinary daidzein, the OR of T2D were 0·81 (95 % CI 0·54, 1·21; P_{trend} = 0·41) for pre-menopausal women, 0·76 (95 % CI 0·52, 1·11; P_{trend} = 0·19) for post-menopausal women with hormone use and 0·58 (95 % CI 0·34, 0·97; P_{trend} = 0·06) for post-menopausal women without hormone use. Consistently, in joint association analysis, the inverse association between urinary daidzein and risk of T2D appeared to be stronger in post-menopausal women without hormone use (Fig. 1(a)). No significant interactions between other metabolites and risk of T2D by menopausal status and hormone use were found. Further stratified analyses were conducted by age, BMI and AHEI, and the inverse association appeared to be more apparent among women with a BMI <30 ($P_{interaction}$ = 0·03) (online Supplementary Table S4).

Discussion

In these two cohorts of US women, urinary excretion of the main isoflavone metabolites, daidzein and genistein, was associated with a lower risk of T2D. Further analyses suggested that inverse association between daidzein and risk of T2D appeared to be stronger among post-menopausal women who did not take hormone therapy at sample collection. These associations were independent of established diabetes risk factors – such as BMI, physical activity and overall diet quality.

Isoflavones are able to bind to the oestrogen receptors (ER), especially ER-β, with 10^3–10^4 less potency than oestradiol[23]. These compounds can exert either oestrogenic or anti-oestrogenic action depending on the level of oestradiol in circulation. When

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Table 1. Age-adjusted baseline characteristics according to diabetes cases and controls in the combined cohort (Medians and interquartile ranges; percentages)

	Cases (*n* 1111)		Controls (*n* 1111)	
Characteristics*	Median	Interquartile range	Median	Interquartile range
Age at urine collection (years)	53·4		53·4	
BMI (kg/m^2)	32·0		25·9	
Physical activity (MET-h/week)	16·4		19·5	
Current smoker (%)	11		7	
Hypertension (%)	38		19	
Hypercholesterolaemia (%)	55		35	
Family history of diabetes (%)	37		21	
First morning urine (%)†	88		88	
Post-menopausal (%)†	83		83	
Post-menopausal hormone use (% of post-menopausal women)†	43		43	
White (%)†	96		97	
Urinary metabolites (nmol/g creatinine)				
Daidzein	311	112, 956	343	123, 1013
Genistein	122	48, 424	128	48, 419
Desmethylangolensin	29	9, 116	36	12, 135
Dihydrodaidzein	34	6, 217	42	9, 288
Dihydrogenistein	25	10, 72	27	12, 78
Diet				
Total energy (kJ/d)	7803		7426	
Total energy (kcal/d)	1865		1775	
Alcohol (g/d)	0·3		0·4	
Coffee (cups/d)	1·0		1·2	
Soft drinks (servings/d)	1·2		0·9	
Tofu consumers (%)	10		12	
Soya milk consumers (%)	4		7	
Fruits (servings/d)	2·0		2·0	
Vegetables (servings/d)	3·5		3·3	
Red meat (servings/d)	1·5		1·3	
Fish (servings/d)	0·2		0·2	
Hot dogs (servings/d)	0·1		0·1	
Alternate Healthy Eating Index score	49		52	

NHS, Nurses' Health Study.
* All of the variables are age-adjusted, except the urinary metabolites of isoflavones. Values of continuous variables are medians. Percentages are based on non-missing data.
† Matching factors; menopausal status and hormone replacement therapy are matching factors for NHSII only.

endogenous oestrogen levels are low, isoflavones primarily exert oestrogen-like effects[32]. Our observation that a stronger inverse association between isoflavones – especially daidzein – and risk of T2D was noted among post-menopausal women without current hormone use is in line with the notion that isoflavones exert oestrogen-like effects on blood glucose when circulating-oestrogen levels are low. In addition, this inverse association was also consistent with the results from short-term clinical trials. Supplementation of isoflavones did not significantly lower fasting glucose or insulin levels in a comprehensive meta-analysis of randomised clinical trials[13]. The non-significant results of those trials might be because of short duration and might not reflect the potential anti-diabetic effect of long-term isoflavone intake. Meanwhile, in another meta-analysis that focused on peri-menopausal and post-menopausal non-Asian women who did not take hormone replacement therapy, isoflavone supplementation significantly lowered fasting insulin and homoeostasis model assessment of insulin resistance levels[33]. Meanwhile, a stronger inverse association between urinary daidzein and risk of T2D was observed among non-obese participants. This association may be related to concentration of blood-circulating oestrogen[34], as adipose tissue is the main source of circulating estrogens for post-menopausal women without hormone use[35].

Besides binding to ER, isoflavones may also bind to and activate nuclear receptors, which regulate lipid and glucose metabolism, including liver X binding receptors, sterol regulated element binding protein, peroxisome-proliferator activated receptors α (PPARα) and PPARγ[36–38]. Isoflavones may also increase the phosphorylation of AMP-activated protein kinase and acetyl-CoA carboxylase to improve glucose up-take and fatty acid oxidation[39]. Animal studies have also shown that isoflavones may improve hyperglycaemia, glucose tolerance and circulating insulin concentrations[40].

Several cohort studies have been conducted to examine the association between intakes of soya foods and isoflavones estimated using FFQ and risk of T2D[15–17,41,42]. For example, in a Japanese population, higher intakes of isoflavones were not associated with diabetes risk in the total study population, but an inverse association was found among overweight women[16]. In the Singapore–Chinese Health Study, intakes of total isoflavones were not associated with T2D risk, probably because many soya foods were sweetened in Singapore[15]. In the EPIC-InterAct Study, total isoflavone intake was not associated with T2D risk among men and women in eight European countries[17]. In addition, two other studies assessed soya food consumption with risk of T2D. An inverse association

Table 2. Odds ratios of type 2 diabetes by tertiles of urinary isoflavones (nmol/g creatinine) in the combined cohort (Odds ratios and 95 % confidence intervals; medians and ranges)

	Tertiles of urinary markers					
		2		3 (highest)		
	1 (lowest)	OR	95 % CI	OR	95 % CI	P_{trend}
Daidzein						
Median	77		326		1529	
Range	0, 171		171, 665		665, 196 860	
Case/control	386/354		369/372		356/385	
Model 1*	1·00	0·88	0·72, 1·07	0·85	0·70, 1·04	0·18
Model 2†	1·00	0·78	0·60, 1·02	0·71	0·55, 0·93	0·03
Genistein						
Median	33		125		729	
Range	0, 65		65, 269		270, 375 579	
Case/control	376/364		370/371		365/376	
Model 1*	1·00	0·94	0·76, 1·15	0·92	0·74, 1·13	0·50
Model 2†	1·00	0·70	0·53, 0·93	0·74	0·56, 0·97	0·15
Desmethylangolensin						
Median	6		33		225	
Range	0, 16		16, 78		78, 160 620	
Case/control	393/347		365/376		353/388	
Model 1*	1·00	0·82	0·68, 1·01	0·77	0·63, 0·94	0·039
Model 2†	1·00	1·00	0·77, 1·31	0·92	0·70, 1·21	0·74
Dihydrodaidzein						
Median	2		38		568	
Range	0, 14		14, 126		126, 61 237	
Case/control	392/348		366/375		353/388	
Model 1*	1·00	0·90	0·73, 1·10	0·78	0·63, 0·97	0·043
Model 2†	1·00	0·94	0·72, 1·23	0·80	0·60, 1·06	0·16
Dihydrogenistein						
Median	7		26		150	
Range	0, 15		15, 49		49, 64 372	
Case/control	389/351		357/384		365/376	
Model 1*	1·00	0·84	0·68, 1·02	0·89	0·72, 1·09	0·55
Model 2†	1·00	0·73	0·56, 0·96	0·82	0·62, 1·08	0·45
Total isoflavones						
Median	198		760		3893	
Range	3, 383		384, 1601		1603, 428 500	
Case/control	379/361		370/371		362/379	
Model 1*	1·00	0·95	0·77, 1·16	0·92	0·75, 1·13	0·50
Model 2†	1·00	0·83	0·63, 1·08	0·80	0·61, 1·05	0·20

NHS, Nurses' Health Study.

* Model 1: conditional logistic model stratified by matching factors, including age at urine sample collection, month of sample collection, first morning urine (yes or no) and race (White or other races) in both cohorts. Menopausal status (pre-menopausal or post-menopausal), and hormone replacement therapy (yes or no) were additionally matched for in NHSII.

† Model 2: conditional logistic model were additionally adjusted for family history of diabetes (yes or no), hypertension at baseline (yes or no), hypercholesterolaemia at baseline (yes or no), BMI (kg/m^2), smoking (non-smoker, past smoker, current smoker), alternative healthy eating index, physical activity (MET-h/week), total energy intake (kJ/d (kcal/d)), menopausal status (pre-menopausal or post-menopausal) and hormone replacement therapy (yes or no).

was found in a Chinese cohort[41], although a positive association was found in the Multi-ethnic Cohort in Hawaii[42]. Differences in processing and cooking methods of soya food might be an explanation for these inconsistent associations. Moreover, measurement errors of FFQ assessments may be of a particular concern, especially among Western populations who consume much less soya foods than do Asians. To our knowledge, no previous prospective studies have examined isoflavone biomarkers in relation to T2D risk. In cross-sectional studies on the association between urinary isoflavones and blood glucose concentration, no associations were found[43]. The lack of association might be because of the reverse causation bias that the diabetes participants might tend to increase consumption of plant-based diet containing soya foods.

Our study had several strengths, including large sample size, long follow-up duration and use of urinary biomarkers to represent the internal dose of isoflavones and to account for the inter-individual variations in bioavailability. Our study also had several limitations. First, as reflected by the low ICC of isoflavone markers in spot urine samples, the urinary biomarkers might not represent long-term excretion of isoflavones well. The low ICC might result from the short half-lives of isoflavone metabolites in the human body, low frequency of consumption, and use of spot urine samples with poor time integration that were collected at various time points after the last meal. In general, non-differential misclassification of the exposure biases the results towards null. Moreover, because of the low reproducibility as well as the measurement errors associated with FFQ assessments of soya foods, only moderate correlation was

Table 3. Stratified analysis of the association between urine isoflavones biomarkers and risk of type 2 diabetes by menopausal status and post-menopausal hormone use in the combined cohort*
(Odds ratios and 95 % confidence intervals)

			T2		T3		
	Number of participants	T1	OR	95 % CI	OR	95 % CI	$P_{interaction}$†
Daidzein							
Pre-menopausal	975	1·00	0·85	0·58, 1·24	0·81	0·54, 1·21	
Post-menopausal with HRT	821	1·00	0·86	0·59, 1·25	0·76	0·52, 1·11	
Post-menopausal without HRT	426	1·00	0·70	0·41, 1·19	0·58	0·34, 0·97	0·001
Genistein							
Pre-menopausal	975	1·00	0·96	0·66, 1·41	1·03	0·70, 1·52	
Post-menopausal with HRT	821	1·00	0·73	0·50, 1·07	0·75	0·52, 1·10	
Post-menopausal without HRT	426	1·00	0·58	0·34, 1·00	0·51	0·30, 0·86	0·36
DMA							
Pre-menopausal	975	1·00	1·19	0·80, 1·77	1·27	0·86, 1·87	
Post-menopausal with HRT	821	1·00	1·06	0·73, 1·53	0·74	0·51, 1·09	
Post-menopausal without HRT	426	1·00	0·79	0·47, 1·34	0·77	0·45, 1·33	0·21
DHDE							
Pre-menopausal	975	1·00	1·22	0·82, 1·80	1·22	0·82, 1·82	
Post-menopausal with HRT	821	1·00	0·99	0·68, 1·43	0·65	0·44, 0·96	
Post-menopausal without HRT	426	1·00	0·75	0·44, 1·28	0·63	0·37, 1·05	0·82
DHGE							
Pre-menopausal	975	1·00	0·70	0·48, 1·03	1·03	0·70, 1·51	
Post-menopausal with HRT	821	1·00	0·84	0·57, 1·23	0·66	0·45, 0·96	
Post-menopausal without HRT	426	1·00	0·80	0·47, 1·36	0·71	0·42, 1·22	0·90

DHDE, dihydrodaidzein; DHGE, dihydrogenistein; DMA, desmethylangolensin; HRT, hormone replacement therapy; NHS, Nurses' Health Study; T, tertile.
* Given that menopausal status and HRT were not matching factors in NHS, unconditional logistic models were used for the stratified analysis and multivariate models adjusted for age at urine sample collection, month of sample collection, fasting status (≥8 h or not), first morning urine (yes or no), race (White or other races), family history of diabetes (yes or no), hypertension at baseline (yes or no), hypercholesterolaemia at baseline (yes or no), BMI (continuous), smoking (non-smoker, past smoker, current smoker), alternative healthy eating index (continuous), physical activity (continuous) and total energy intake (continuous).
† Likelihood ratio test comparing the conditional logistic models with and without interaction term was used; the urine isoflavones were dichotomised and the pre-menopausal and post-menopausal with HRT were combined into one group.

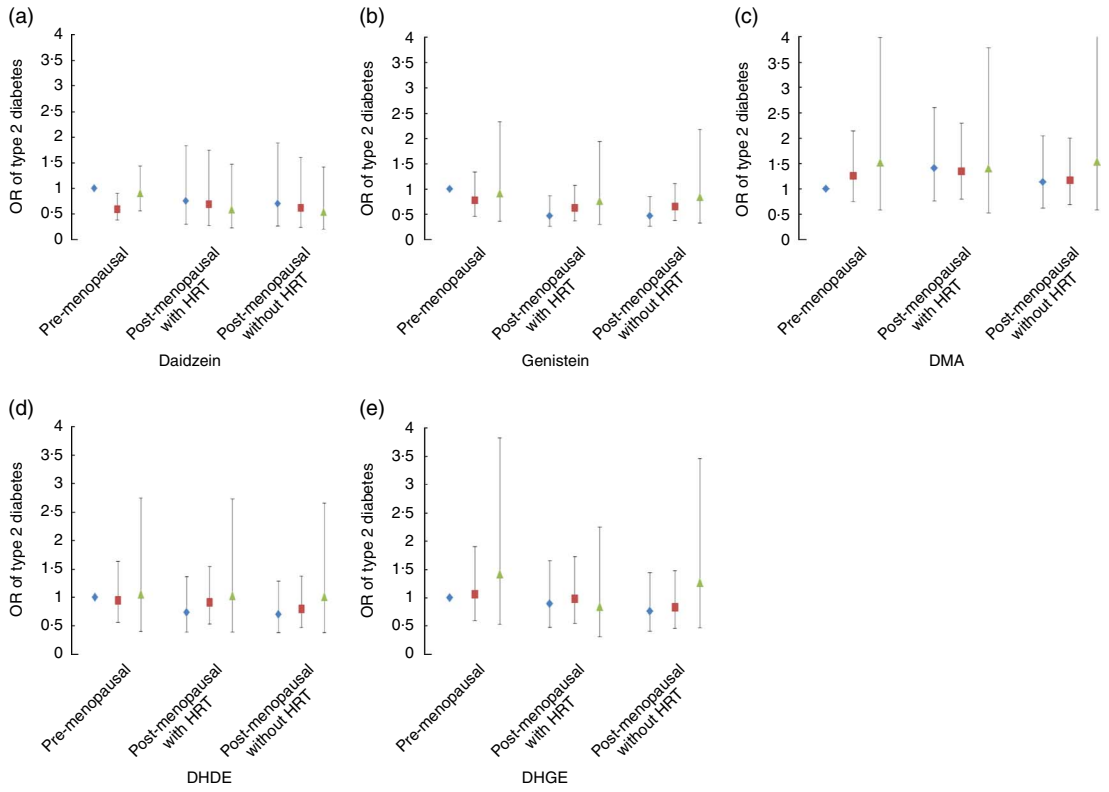

Fig. 1. Joint association of urinary isoflavone biomarkers and post-menopausal status and hormone use with odds of type 2 diabetes. Conditional logistic models are adjusted for hypertension at baseline (yes or no), hypercholesterolaemia at baseline (yes or no), BMI (kg/m^2), smoking (non-smoker, past smoker, current smoker), alternative healthy eating index, physical activity (MET-h/week) and total energy intake (kJ/d (kcal/d)). DHDE, dihydrodaidzein; DHGE, dihydrogenistein; DMA, desmethylangolensin; HRT, hormone replacement therapy. ◆, Lowest tertile; ■, second lowest tertile; ▲, highest tertile.

found between soya foods assessed by FFQ and urinary isoflavones, indicating that urinary isoflavones might not represent long-term relatively low soya food consumption well. Meanwhile, given the between-person variability in isoflavones' bioavailability partially due to microbiota processing and other physiological processes during absorption and metabolism[44], FFQ assessments of isoflavones and soya foods may not measure exposures to these isoflavone metabolites well. Second, although we adjusted for an array of established and potential risk factors of T2D, we could not exclude the possibility that un-measured confounding or residual confounding, such as a healthy lifestyle, might still partially explain the association between isoflavones and risk of T2D. Third, we cannot exclude the possibility that chance may play a role in our findings, especially when the inverse association was primarily observed for daidzein. Fourth, we did not observe significant association between total urinary isoflavones and risk of T2D. Given that these individual metabolites have different levels of measurement errors, different time integration (as reflected by ICC) and different levels of microbiota processing, the associations for total isoflavones may be prone to attenuation towards the null due to this heterogeneity. Lastly, the observed associations may have limited generalisability within White health professionals. In addition, the urinary excretion of isoflavones was somehow lower than that was observed in NHANES (mean urinary isoflavones: 4·9 *v.* 149·1 μg/g creatine in our study), further limiting the generalisability to populations with high levels of isoflavone intake.

In conclusion, we observed inverse associations between urinary excretion of daidzein and genistein and risk of T2D in US women. In addition, the inverse association for daidzein was stronger among post-menopausal women who did not use hormone replacement therapy. Although these findings are in line with evidence from animal experiments and clinical trials demonstrating benefits of isoflavone intake on insulin resistance, further studies are warranted to replicate the current findings among other western populations with low isoflavone intake levels. In addition, multiple-day 24 h urine samples shall be used to achieve more stable estimates of isoflavone-marker excretion.

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Q. S., A. A. F., F. B. H. and R. Mv. D. were involved in data collection of dietary flavonoids or urinary metabolites. A. A. F. measured urinary metabolites using liquid chromatography MS. Q. S. and R. Mv. D. conducted pilot studies for the current investigation. Q. S., F. B. H., B. A. R. and R. Mv. D. provided statistical expertise. M. D. analysed the data and wrote the first draft of the manuscript. Q. S., F. B. H., A. A. F., S. S. T., B. A. R. and R. Mv. D. contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. Q. S. is the guarantor of this investigation.

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Supplementary material

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