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Urinary enterolactone is associated with obesity and metabolic alteration in men in the US National Health and Nutrition Examination Survey 2001–10

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Abstract

Phyto-oestrogens are a family of plant-derived xeno-oestrogens that have been shown to prevent cancer in some studies. Whether phyto-oestrogen intake affects obesity status in a population is still unclear. In the present cross-sectional study, we examined the association of urinary phyto-oestrogen metabolites with obesity and metabolic parameters in children and adults. Data from 1294 children (age 6–19 years) and from 3661 adults (age ≥20 years) who participated in the US National Health and Nutrition Examination Survey 2001–10 were analysed. Multivariate logistic regression was applied to investigate the associations of BMI, waist circumference, serum metabolites (total cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, fasting glucose and fasting insulin) and the metabolic syndrome with urinary phyto-oestrogen levels. When stratified by age and sex, we found a stronger association (OR 0·30, 95% CI 0·17, 0·54; $P<0·001$) between urinary enterolactone levels and obesity in adult males (age 20–60 years) than in children (age 12–19 years) or the elderly (age >60 years) in the same survey. However, no associations with urinary daidzein, *O*-desmethylangolensin, equol, enterodiol or genistein were found in the overall population. We also found that the elevation of enterolactone levels was inversely associated with TAG levels, fasting glucose levels, fasting insulin levels and the metabolic syndrome in males aged 20–60 years, but positively associated with HDL-cholesterol levels. The present results provide epidemiological evidence that urinary enterolactone is inversely associated with obesity in adult males.

Key words: Phyto-oestrogens: Enterolactone: Obesity: Metabolic parameters: National Health and Nutrition Examination Survey

Obesity is highly prevalent in the USA. Its incidence in adults was 13% in 1962 and climbed to 34·9% in 2010, as reported by the Centers for Disease Control and Prevention[1]. Moreover, in 2011–2, 16·9% of American children and adolescents were obese, which was a serious public health problem[1]. Obesity can cause several co-morbidities, such as CVD and respiratory disease, type 2 diabetes, certain cancers, and the metabolic syndrome[2,3]. Approximately 50% of overweight adults have already been reported to be overweight in childhood, and most obese children remain obese as adults[4]. Obesity is most commonly caused by a combination of genetic susceptibility, unhealthy behaviours and harmful environmental factors[5]. Previous studies have linked environmental endocrine-disrupting chemicals such as diethylstilboestrol, bisphenol A, phyto-oestrogens, phthalates and organotins with adverse health consequences, including obesity and diabetes[6].

Phyto-oestrogens, a family of plant-derived xeno-oestrogens, include two main forms, isoflavones and lignans, both of which are common in the human diet. Due to their structural similarity with oestradiol, they can cause oestrogenic and/or anti-oestrogenic effects in organisms[7]. Phyto-oestrogens have been used to prevent postmenopausal bone loss[8]. However, the biological effect of phyto-oestrogens remains unclear. Several studies have reported that phyto-oestrogen administration is protective against breast and prostate cancers and CVD[9]. Phyto-oestrogens cannot be considered nutrients because no characteristic deficiency syndrome is caused by

Abbreviations: NHANES, National Health and Nutrition Examination Survey; PIR, poverty:income ratio.

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their absence from the diet, and they do not cause any essential physiological malfunction.

To our knowledge, few data on the relationship between phyto-oestrogens and obesity in human subjects have been reported. Several studies that used National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2004 have suggested that urinary enterolignan concentrations were significantly associated with serum TAG levels, HDL-cholesterol levels[10] and the presence of the metabolic syndrome[11]. However, the data were not stratified by age and sex, which is necessary because phyto-oestrogens have shown activities similar to oestradiol. In addition, the sample size was not large. Therefore, in the present study, we investigated the association of urinary phyto-oestrogens with obesity and metabolism-related indicators such as TAG levels, insulin levels and the metabolic syndrome in adults aged 20–60 years. The results were obtained from the NHANES 2001–10 and stratified by sex.

Materials and methods

Study population

The NHANES studies were conducted by the US National Center for Health Statistics (Centers for Disease Control and Prevention, Atlanta, GA, USA). The NHANES is a cross-sectional study that used a stratified, multi-stage sample, representative of non-institutionalised civilians in the USA, which was approved by the National Center for Health Statistics Research Ethics Review Board. The subjects enrolled in the present study were obtained from five cycles of the NHANES (2001–2, 2003–4, 2005–6, 2007–8 and 2009–10). Interviews and substantial physical examinations, which included blood and urine collection, were performed in the Mobile Examination Centers. We examined the association of urinary phyto-oestrogens with obesity and metabolism-related indicators in children and adults who participated in the NHANES.

Participants in the NHANES over a range of 10 years were selected to form a random subgroup for the measurement of urinary phyto-oestrogen and serum metabolite levels. Pregnant women (n 383) were excluded from the study because they are considered to have an abnormal physiological status that prevents the accuracy of the original BMI. Women who had undergone an ovariectomy (n 74) were also excluded. The final study sample consisted of 1294 children (6–19 years of age, 694 males and 600 females) and 3661 adults (≥20 years of age, 1851 males and 1810 females) from this subgroup.

Evaluation of fatness: BMI and waist circumference

BMI was computed as weight divided by height squared (kg/m^2). Waist circumference was measured by professionals using a standardised protocol. Among children, individuals were classified as being overweight or obese according to cut-off points based on sex and age[12]. Among adults, subjects were classified as overweight if they had a BMI value ranging between 25 and 30 kg/m^2, and obese if they had a BMI ≥30 kg/m^2. For waist circumference, the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) recommends a single set of sex-specific cut-offs: >102 cm for men and >88 cm for women[13].

The metabolic syndrome

The metabolic syndrome was diagnosed by incorporating the International Diabetes Federation and AHA/NHLBI definitions from 2009[14]. In brief, the diagnosis of the metabolic syndrome includes any three of the following five criteria: elevated waist circumference (according to population- and country-specific cut-offs: ≥102 cm (men) or ≥88 cm (women) in the USA); serum TAG level >1·70 mmol/l (≥150 mg/dl); serum HDL-cholesterol level <1·03 mmol/l (<40 mg/dl) (men) or <1·30 mmol/l (<50 mg/dl) (women); systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg; fasting plasma glucose level ≥5·55 mmol/l (≥100 mg/dl).

Laboratory analysis

Phyto-oestrogen metabolites were measured in spot urine samples from participants aged ≥6 years. Spot urine samples were obtained in collection cups at the Mobile Examination Centers and were quickly frozen at −20°C. Phyto-oestrogen concentration was measured by two different methods. In the survey years 2001–2, phyto-oestrogen samples were analysed by a Sciex API III heated nebulizer–atmospheric pressure chemical ionisation interface coupled with MS/MS[15]. In the following years, the samples were analysed by HPLC–atmospheric pressure photoionisation–tandem MS[16] (see Laboratory procedure manual available at http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/Phyto_F_met_phytoestrogens.pdf). The two methods are comparable, and both incorporated enzymatic deglucuronidation before a solid-phase extraction was conducted. Subsequently, the components were resolved by reverse-phase HPLC. MS with internal isotope-labelled standards was used to ensure proper accuracy and detection limit. No differences between the two HPLC methods for urinary phyto-oestrogens were reported in the NHANES protocols, and there were no biologically significant differences (NHANES 1999–2002 $v.$ 2003–4)[10]. Information on other covariates, such as age, sex, race/ethnicity, education level, and poverty:income ratio (PIR), was obtained from a standardised questionnaire.

Covariates

The PIR is a measure of socio-economic status and defined as the calculated ratio of family income to the poverty threshold after adjusting for inflation and family size. Mean dietary fibre intake and energy intake values were calculated from 2 d dietary interviews. Energy intake was classified as 'normal' or 'excessive' based on the US Department of Agriculture[17] guidelines, stratified by age and sex. Urinary creatinine, a measure of urinary dilution, was included in the analyses as an independent variable. Serum cotinine, a marker of

exposure to environmental cigarette smoke, was measured and divided into quartiles. Serum total cholesterol, serum LDL-cholesterol and fasting insulin were divided into higher weighted (≥90 %) and lower weighted (<90 %) values. TAG level >1·70 mmol/l (≥150 mg/dl) and the use of a cholesterol-lowering medication, serum HDL-cholesterol level <1·03 mmol/l (<40 mg/dl) (men) or <1·30 mmol/l (<50 mg/dl) (women), and fasting plasma glucose level ≥5·55 mmol/l (≥100 mg/dl) were classified according to the International Diabetes Federation and AHA/NHLBI guidelines.

Statistical analyses

We used multinomial logistic regression models to estimate adjusted OR with 95 % CI for the association of obesity and overweight status and waist circumference as distinct outcomes (*v.* normal/underweight) with categorical phyto-oestrogen exposure. The lowest quartile (quartile 1) was used as the reference value. As age and sex are extremely important variables, we conducted separate analyses stratified by age (6–19, 20–60 and >60 years) and sex. We controlled for the following *a priori* confounding factors for the associations of phyto-oestrogens with BMI and waist circumference: age; race/ethnicity; energy intake; dietary fibre intake; PIR; serum cotinine level; urinary creatinine level; education level (in adults). We also adjusted for age, dietary fibre intake, urinary creatinine level and serum cotinine level to explore the relationship between phyto-oestrogens and serum metabolites. All statistical analyses were performed using the Statistical Analysis Systems software package version 9.2 (SAS Institute, Inc.). A P value <0·05 was designated as the cut-off for statistical significance.

Table 1. Weighted characteristics of the study participants in the NHANES (National Health and Nutrition Examination Survey) 2001–10*
(Mean values with their standard errors)

	12–19 years				20–60 years				>60 years			
	Male (*n* 694)		Female (*n* 600)		Male (*n* 1273)		Female (*n* 1226)		Male (*n* 578)		Female (*n* 584)	
Characteristics	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Daidzein (ng/ml)	471	64·6	369	38·4	426	54·7	404	46·6	283	35·7	247	32·3
O-Desmethylangolensin (ng/ml)	107	30·8	74·1	15·7	98·1	22·1	142	24·3	77·7	22·0	132	42·2
Equol (ng/ml)	46·1	9·63	61·1	25·5	36·7	8·22	87·1	23·4	38·5	10·7	34·9	10·4
Enterodiol (ng/ml)	84·0	5·93	140	32·9	167	24·2	184	34·0	161	31·2	117	11·3
Enterolactone (ng/ml)	670	37·33	781	90·7	880	89·2	990	132	1089	66·0	867	57·8
Genistein (ng/ml)	185	29·32	140	16·6	194	29·5	159	16·1	159	23·9	147	21·8
Age (years)	15·6	0·09	15·4	0·09	40·2	0·33	40·0	0·33	71·2	0·29	71·9	0·31
BMI (kg/m^2)	23·4	0·21	24·0	0·25	28·4	0·18	29·4	0·22	28·6	0·21	29·3	0·26
C-reactive protein (mg/l)	1·7	0·2	1·7	0·1	3·4	0·2	5·3	0·3	4·9	0·4	5·2	0·3
Waist circumference (cm)	NA	NA	NA	NA	98·9	0·43	95·2	0·47	105	0·56	99·3	0·60
Blood cotinine (ng/ml)	16·3	2·30	15·9	2·68	84·6	4·45	60·6	4·08	38·1	4·80	30·5	4·73
Urinary creatinine												
mmol/l	14	0·29	13	0·31	14	0·21	11	0·17	11	0·24	8	0·19
mg/dl	164	3·33	146	3·51	161	2·42	120	1·97	127	2·71	90·1	2·16
Dietary fibre (g)	15·7	0·33	12·1	0·28	17·8	0·31	14·3	0·22	17·2	0·40	14·6	0·32
Race (%)												
Mexican American	31·0		29·2		22·6		22·3		14·5		16·4	
Other Hispanic	6·2		6·2		7·1		7·9		6·4		5·0	
Non-Hispanic White	29·5		29·0		45·9		43·6		61·9		60·1	
Non-Hispanic Black	29·0		30·8		20·0		21·0		15·2		15·9	
Other	4·3		4·8		4·4		5·2		1·9		2·6	
Weight (%)												
Normal and underweight	60·2		62·0		29·0		32·5		23·4		26·2	
Overweight	23·9		18·8		38·6		28·0		41·3		32·4	
Obese	14·1		17·8		31·3		38·7		32·4		38·9	
Television, video game and computer usage (%)												
≤2 h	15·0		17·2		8·6		9·1		3·1		4·5	
>2 h	41·9		40·8		28·1		24·6		32·2		31·0	
Energy intake (%)												
Normal intake	55·5		58·5		48·5		56·9		69·7		68·2	
Excessive intake	32·9		33·3		36·5		31·1		20·8		22·4	
PIR												
≤1	29·1		31·5		17·0		19·2		12·8		15·6	
>1	65·3		62·2		76·8		73·5		78·2		75·3	
Education level (%)												
Below high school	NA		NA		27·6		24·8		35·1		36·0	
High school	NA		NA		26·2		20·1		24·0		27·1	
Above high school	NA		NA		46·3		55·1		40·8		37·0	

PIR, poverty:income ratio; NA, not applicable.
* Participants were excluded if they had a positive laboratory pregnancy test or self-reported to be pregnant at exam (*n* 383), and had undergone an ovariectomy (*n* 74).

Results

General characteristics

Table 1 presents the baseline characteristics and mean concentrations of urinary phyto-oestrogen metabolite levels among the participants (12–19, 20–60 and >60 years) included in the present study from the NHANES 2001–10 database. The response rates for daily hours of television, video games and computer use were approximately 50 %, so they were not included in the present analysis. Table 2 presents the serum metabolite levels of the participants.

Overweight, obesity and high-risk waist circumference

As a whole, the adjusted OR from the multinomial logistic regression models adjusted for factors such as age, race/ethnicity, urinary creatinine, dietary fibre intake, PIR, serum cotinine level, energy intake and education level (in adults) for enterolactone are presented in Table 3. Data for daidzein, *O*-desmethylangolensin, equol, enterodiol genistein are summarised in online supplementary Table S1. Furthermore, the association between BMI and quartiles of urinary enterolactone in men is shown in Fig. 1. The present results showed strong inverse associations with obesity and waist circumference for enterolactone in men aged 20–60 years. Notably, the highest concentration of enterolactone was associated with obesity status (OR 0·30, 95 % CI 0·17, 0·54; $P<0·001$) and high-risk waist circumference (OR 0·32, 95 % CI 0·21, 0·51; $P<0·001$) in 20- to 60-year-olds. However, in women, the highest concentration of enterolactone was only inversely associated with overweight status (OR 0·43, 95 % CI 0·24, 0·77; $P=0·005$).

Serum metabolites and the metabolic syndrome

Because obesity status increases the risk of several physical conditions, we further explored the association between some serum profiles and the metabolic syndrome for enterolactone that exerts potential actions in 20- to 60-year-olds. The results are shown in Table 4 and positive results are shown in Fig. 2. Higher enterolactone levels had a stronger relationship with HDL-cholesterol levels, TAG levels, fasting glucose levels and fasting insulin levels, particularly in men. In addition, the highest concentration of enterolactone was linked to a lower risk of the metabolic syndrome in men (OR 0·46, 95 % CI 0·30, 0·70; $P<0·001$).

Discussion

In a large-scale, nationally representative sample of US children and adults, we found independent associations between various levels of urinary enterolactone and obesity, and high-risk waist circumference. These associations were significant in men aged 20–60 years. Further analysis of serum biochemical parameters showed negative associations between urinary enterolactone levels, serum TAG levels, fasting glucose levels and fasting insulin levels, and a positive association with HDL-cholesterol. Interestingly, higher urinary enterolactone levels correlated with a lower risk of the metabolic syndrome in men aged 20–60 years.

Table 2. Serum metabolites of the study participants aged 20–60 years (Mean values with their standard errors)

	12–19 years				20–60 years				>60 years			
	Male (*n* 694)		Female (*n* 600)		Male (*n* 1273)		Female (*n* 1226)		Male (*n* 578)		Female (*n* 584)	
Characteristics	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total cholesterol												
mmol/l	4·1	0·03	4·2	0·03	5·2	0·03	5·0	0·03	4·9	0·04	5·3	0·04
mg/dl	158	1·18	162	1·18	199	1·23	194	1·18	190	1·67	205	1·66
HDL-cholesterol												
mmol/l	1·3	0·01	1·4	0·01	1·2	0·01	1·5	0·01	1·3	0·01	1·6	0·02
mg/dl	51·0	0·47	54·5	0·52	48·2	0·40	56·7	0·44	49·0	0·57	60·9	0·70
LDL-cholesterol												
mmol/l	2·3	0·03	2·3	0·03	3·1	0·03	2·9	0·02	2·9	0·04	3·0	0·04
mg/dl	89·8	1·10	90·4	1·00	120	1·01	113	0·96	112	1·47	116	1·48
TAG												
mmol/l	1·0	0·02	0·9	0·02	1·9	0·07	1·4	0·03	1·6	0·04	1·6	0·04
mg/dl	86·2	1·95	81·4	1·72	166	6·27	121	2·48	144	3·54	143	3·25
Fasting glucose												
mmol/l	5·3	0·03	5·1	0·05	5·8	0·05	5·6	0·05	6·4	0·08	6·3	0·09
mg/dl	94·8	0·61	91·7	0·87	105	0·96	100	0·88	116	1·46	114	1·60
Fasting insulin												
pmol/l	86·1	2·50	102·1	3·26	91·7	2·78	86·1	2·01	93·8	3·89	95·1	3·82
μIU/ml	12·4	0·36	14·7	0·47	13·2	0·40	12·4	0·29	13·5	0·56	13·7	0·55
Systolic blood pressure (mmHg)	NA	NA	NA	NA	118	0·62	119	0·67	119	1·02	119	0·96
Diastolic blood pressure (mmHg)	NA	NA	NA	NA	65·4	0·49	66·2	0·50	66·9	0·67	67·6	0·74
Metabolic syndrome (%)	NA		NA		34·2		28·2		53·6		56·0	

NA, not applicable.

Table 3. OR for the association between the quartiles of urinary enterolactone and overweight, obesity and high-risk waist circumference *v.* normal/underweight and low risk*
(Odds ratios and 95 % confidence intervals)

		12–19 years								20–60 years								>60 years							
		Obese† *v.* normal			Overweight† *v.* normal			Obese *v.* normal (≥30 *v.* <25 kg/m²)			Overweight *v.* normal (25–30 *v.* <25 kg/m²)			High-risk *v.* low-risk waist circumference			Obese *v.* normal (≥30 *v.* <25 kg/m²)			Overweight *v.* normal (25–30 *v.* <25 kg/m²)		
Phyto-oestrogens	Sex	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Enterolactone	Male																						
Q1‡		Reference			Reference			Reference			Reference			Reference			Reference			Reference		
Q2‡		0·54	0·25, 1·15	0·11	0·63	0·32, 1·28	0·20	0·70	0·41, 1·21	0·20	0·64	0·38, 1·09	0·10	0·64	0·42, 0·97	0·04	0·71	0·25, 2·05	0·53	0·64	0·22, 1·88	0·41
Q3‡		0·44	0·18, 1·08	0·81	1·10	0·56, 2·17	0·79	0·56	0·33, 0·95	0·03	0·50	0·30, 0·84	0·01	0·57	0·38, 0·87	0·01	1·07	0·39, 2·98	0·90	1·12	0·40, 3·09	0·83
Q4‡		0·30	0·12, 0·76	0·01	0·76	0·36, 1·59	0·46	0·30	0·17, 0·54	<0·001	0·67	0·40, 1·12	0·12	0·32	0·21, 0·51	<0·001	0·62	0·23, 1·70	0·36	0·67	0·25, 1·77	0·42
P for trend				0·05			0·97			0·008			0·25			0·001			0·18			0·37
Enterolactone	Female																						
Q1‡		Reference			Reference			Reference			Reference			Reference			Reference			Reference		
Q2‡		0·53	0·23, 1·23	0·14	1·85	0·82, 4·18	0·14	0·51	0·30, 0·87	0·01	0·62	0·35, 1·12	0·11	0·64	0·41, 0·99	0·05	0·49	0·21, 1·18	0·11	1·03	0·42, 2·52	0·95
Q3‡		0·73	0·34, 1·61	0·44	0·88	0·36, 2·18	0·79	0·42	0·25, 0·71	<0·001	0·53	0·29, 0·95	0·03	0·57	0·36, 0·90	0·02	0·38	0·15, 0·96	0·04	1·31	0·53, 3·25	0·55
Q4‡		0·41	0·16, 1·08	0·07	1·13	0·44, 2·94	0·80	0·35	0·21, 0·60	<0·001	0·43	0·24, 0·77	0·005	0·44	0·28, 0·68	<0·001	0·23	0·09, 0·60	0·002	0·88	0·36, 2·14	0·77
P for trend				0·38			0·57			0·06			0·003			0·94			0·07			0·90

* Multinomial logistic regression models were used, adjusted for age, race/ethnicity, urinary creatinine, dietary fibre intake, poverty:income ratio, serum cotinine, energy intake and education level (in adults).
† BMI cut-offs based on age and sex.
‡ Quartiles (Q) of enterolactone (ng/ml): Q1: ≤ 127; Q2: 127–420; Q3: 420–979; Q4: > 979.

Previously, only a few studies have investigated the association between phyto-oestrogen and metabolic profiles. For example, one small population study conducted in 155 Canadian women showed that women with high enterolactone levels have greater insulin sensitivity[18], which is inconsistent with our findings. In another study conducted in 299 pregnant American women, circulating fasting glucose concentrations have been shown to be inversely associated with urinary total isoflavone level[19]. Furthermore, a randomised controlled trial has found that daidzein administration may aid in the reduction of LDL-cholesterol levels[20]. A larger population study that included 1748 participants from the NHANES showed that plasma TAG levels were lower in participants in the highest enterolactone quartile[11]. A cross-sectional analysis of 1492 adults has found that enterolignan levels were associated with higher HDL-cholesterol concentrations and lower serum TAG concentrations in American adults[10]. In the present study, we analysed the whole urinary phyto-oestrogen levels measured by the NHANES. Using these data, we were the first to find an association between urinary enterolactone levels and obesity in males in a cross-sectional study of 1273 adult males aged 20–60 years. Moreover, we showed that an elevation of enterolactone levels was inversely associated with TAG levels, fasting insulin levels and the metabolic syndrome, but positively associated with HDL-cholesterol levels in adult males.

Several studies on the association between phyto-oestrogens and obesity have been conducted. In a recent epidemiological study, *O*-desmethylangolensin, the metabolite of daidzein, has been shown to be negatively associated with obesity[21]. Daidzein, one of the most abundant phyto-oestrogens in the human diet, can regulate lipid and carbohydrate homeostasis and could have health benefits related to human obesity[22,23]. Bhathena & Velasquez[24] proposed that dietary phyto-oestrogens have a beneficial role in obesity. On the contrary, we did not find that daidzein was significantly associated with obesity. As expected, we showed a negative association of another phyto-oestrogen (enterolactone) with obesity in men and of overweight in women. Other phyto-oestrogens such as isoflavone have been reported to affect central appetite mechanisms[25]. A related

Fig. 1. BMI trend for the quartiles of urinary enterolactone levels in men aged 20–60 years in the NHANES (National Health and Nutrition Examination Survey) 2001–10. Values are means, with their standard errors represented by vertical bars.

British Journal of Nutrition

Table 4. OR for the association between the quartiles of urinary enterolactone and serum metabolites of adults aged 20–60 years*
(Odds ratios and 95 % confidence intervals)

Phyto-oestrogens	Sex	Total cholesterol			TAG			HDL-cholesterol			LDL-cholesterol			Fasting blood-glucose			Fasting insulin			Metabolic syndrome		
		OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Enterolactone	Male																					
Q1†		Reference			Reference			Reference			Reference			Reference			Reference			Reference		
Q2†		1·03	0·55, 1·91	0·93	0·79	0·54, 1·16	0·23	1·36	0·91, 2·04	0·14	1·00	0·51, 1·96	0·99	1·09	0·74, 1·62	0·65	0·79	0·45, 1·41	0·43	0·99	0·66, 1·48	0·95
Q3†		0·93	0·50, 1·72	0·82	0·51	0·35, 0·76	0·001	1·72	1·14, 2·59	0·01	1·29	0·70, 2·40	0·42	0·89	0·61, 1·32	0·57	0·57	0·31, 1·05	0·07	0·94	0·63, 1·40	0·77
Q4†		0·75	0·40, 1·43	0·39	0·28	0·18, 0·43	<0·001	3·12	1·97, 4·96	<0·001	1·34	0·72, 2·52	0·36	0·63	0·42, 0·93	0·02	0·36	0·18, 0·72	0·004	0·46	0·30, 0·70	<0·001
P for trend				0·11			0·001			<0·001			0·69			0·001			0·004			<0·001
Enterolactone	Female																					
Q1†		Reference			Reference			Reference			Reference			Reference			Reference			Reference		
Q2†		1·24	0·63, 2·44	0·53	0·92	0·61, 1·39	0·68	0·89	0·61, 1·29	0·53	1·28	0·63, 2·59	0·50	0·83	0·54, 1·26	0·37	0·51	0·27, 0·93	0·03	0·57	0·37, 0·86	0·01
Q3†		1·02	0·50, 2·08	0·96	0·47	0·29, 0·75	0·002	1·32	0·88, 1·97	0·18	0·96	0·44, 2·07	0·91	0·72	0·47, 1·12	0·15	0·47	0·25, 0·89	0·02	0·55	0·36, 0·84	0·01
Q4†		1·69	0·88, 3·25	0·11	0·59	0·38, 0·91	0·02	1·61	1·08, 2·40	0·02	1·83	0·92, 3·63	0·09	0·61	0·39, 0·94	0·02	0·40	0·21, 0·76	0·01	0·51	0·33, 0·77	0·002
P for trend				0·23			0·52			0·38			0·16			0·68			0·73			0·51

*Multinomial logistic regression models were used, adjusted for age, urinary creatinine, dietary fibre intake, serum cotinine. Serum total cholesterol, serum LDL-cholesterol and fasting insulin were divided into higher weighted (≥ 90 %) and lower weighted (< 90 %) values. TAG level > 1·70 mmol/l (≥ 150 mg/dl) and the use of a cholesterol-lowering medication, serum HDL-cholesterol level < 1·03 mmol/l (< 40 mg/dl) (men) or < 1·30 mmol/l (< 50 mg/dl) (women), fasting plasma glucose level ≥ 5·55 mmol/l (≥ 100 mg/dl) were classified according to the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute.
†Quartiles (Q) of enterolactone (ng/ml): Q1: ≤ 127; Q2:127–420; Q3: 420–979; Q4: > 979.

study has also reported that lower doses of genistein are adipogenic and that pharmacological doses of genistein inhibit adipose deposition[26].

The potential mechanism for the association between phyto-oestrogens and obesity has not been fully elucidated. Endocrine disrupters interact with other factors that affect fetal and postnatal growth and can cause obesity[27,28]. These disrupters might play roles in multiple pathways[29]: (1) metabolic sensors, such as the PPAR and the 9-*cis* retinoic acid receptor; (2) sex-steroid receptors; (3) the hypothalamic–pituitary adrenal axis; (4) epigenetic process. Some phyto-oestrogens can act as endocrine disrupters by influencing oestrogen biosynthesis[30]. These include isoflavones, lignans, stilbenes and coumestans. Among the six phyto-oestrogens included in the present study, four were isoflavones (daidzein, *O*-desmethylangolensin, equol, and genistein) and two were lignans (enterodiol and enterolactone)[31]. An *in vivo* study by Cederroth *et al.*[32] discovered that soya-derived phyto-oestrogens have promoting effects on obesity in mice. Furthermore, Taxvig *et al.*[33] found that phyto-oestrogens have an inhibitory effect on adipose cell differentiation *in vitro*, and this phenomenon did not seem to be entirely the result of PPARγ activation/inhibition. Phyto-oestrogens may activate the AMPK pathway, subsequently mediating the advantageous effects in peripheral tissues[34]. This may explain the structural similarities of phyto-oestrogens with endogenous oestrogens. Phyto-oestrogens are able to compete with 17β-oestradiol by binding to the intranuclear oestrogen receptors in different tissues, subsequently causing weak oestrogenic effects[22].

Phyto-oestrogens are ingested as precursors and then metabolised in the gut by the intestinal flora[35]. Thus, different dietary structures and the diverse composition and abundance of the gut microbiota can result in a variety of circulating phyto-oestrogens in the human body, which have a variety of biological functions. A large number of studies have reported that obesity is associated with the composition of the gut microbiota[36]. This could be another explanation contributing to the underlying mechanisms of obesity. However, the present study had some limitations. First, the different ethnicities of the study subjects prevented us from comparing the results of the present study with previous ones. Second, some confounders, such as physical activity level and genetic susceptibility, may influence the associations in the study. In addition to the common covariants, we analysed other covariants that may affect the development of obesity, such as PIR, education level (in adults), and blood cotinine level; performing this covariant analysis ensured that the present results reflected reality. Because of the cross-sectional nature of the present study, it is not possible to estimate whether phyto-oestrogens affected obesity or vice versa. However, some evidence from *in vivo* and *in vitro* studies has suggested that phyto-oestrogen administration may prevent obesity by inhibiting adipocyte differentiation and promoting the apoptosis of mature adipocytes[34,37].

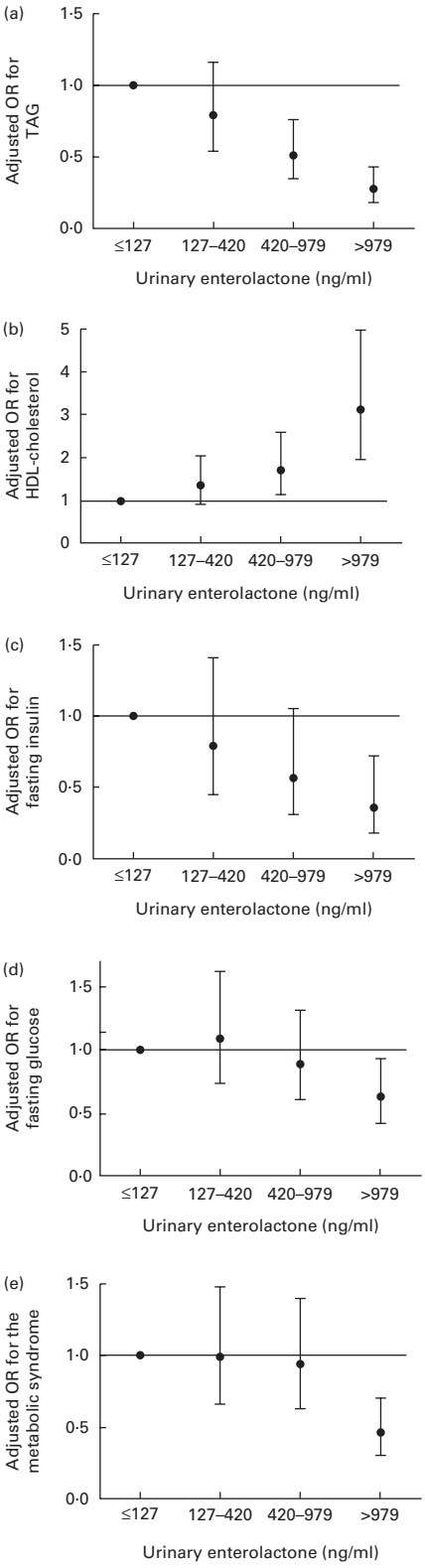

Fig. 2. Adjusted OR for (a) serum TAG levels (P for trend=0·001), (b) HDL-cholesterol levels (P for trend<0·001), (c) fasting insulin levels (P for trend=0·004), (d) fasting glucose levels (P for trend=0·001) and (e) the metabolic syndrome (P for trend<0·001) by increasing quartiles of urinary enterolactone levels in men aged 20–60 years. Values are OR, with 95 % CI represented by vertical bars, adjusted for age, urinary creatinine, dietary fibre intake and serum cotinine.

Conclusion

We found that urinary enterolactone was strongly associated with obesity, waist circumference, serum TAG level, fasting glucose level and fasting insulin level, and positively associated with HDL-cholesterol in 20–60-year-old American males. Most of these factors are key components of the metabolic syndrome. Interestingly, we also found a negative association between enterolactone with the metabolic syndrome in men only. Although the exact mechanism of how phyto-oestrogen contributes to obesity remains unclear, the present results may contribute to a better understanding of obesity from an epidemiological standpoint.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514004115

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The authors' contributions are as follows: Q. L. and C. X. conducted the data analyses; Z.-Y. J. and A. G. were involved in the design of the study and interpretation of the results, and critically reviewed the manuscript. All authors approved the final manuscript.

The authors declare that they have no competing interests.

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