# Variations in Plasma Phytoestrogen Concentrations in European Adults[1,2]

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## Abstract

Dietary phytoestrogens may play a role in chronic disease occurrence. The aim of our study was to assess the variability of plasma concentrations in European populations. We included 15 geographical regions in 9 European countries (Denmark, France, Germany, Greece, Italy, Spain, Sweden, The Netherlands, and UK) and a 16th region, Oxford, UK, where participants were recruited from among vegans and vegetarians. All subjects were participants of the European Prospective Investigation into Cancer and Nutrition (EPIC). Plasma concentrations of 3 isoflavones (daidzein, genistein, and glycitein), 2 metabolites of daidzein [O-desmethylangolensin (O-DMA) and equol] and 2 mammalian lignans (enterodiol and enterolactone) were measured in 1414 participants. We computed geometric means for each region and used multivariate regression analysis to assess the influence of region, adjusted for gender, age, BMI, alcohol intake, smoking status, and laboratory batch. Many subjects had concentrations below the detection limit [0.1 $\mu$g/L (0.4 nmol/L)] for glycitein (80%), O-DMA (73%) and equol (62%). Excluding subjects from Oxford, UK, the highest concentrations of isoflavones were in subjects from the Netherlands and Cambridge, UK [2–6 $\mu$g/L (7–24 nmol/L); $P < 0.05$], whereas concentrations for lignans were highest in Denmark [8 $\mu$g/L (27 nmol/L); $P < 0.05$]. Isoflavones varied 8- to 13-fold, whereas lignans varied 4-fold. In the vegetarian/vegan cohort of Oxford, concentrations of isoflavones were 5–50 times higher than in nonvegetarian regions. Region was the most important determinant of plasma concentrations for all 7 phytoestrogens. Despite the fact that plasma concentrations of phytoestrogens in Europe were low compared with Asian populations, they varied substantially among subjects from the 16 different regions.

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## Introduction

Phytoestrogens are widespread plant compounds that have been proposed to interfere with the occurrence of hormone-dependent cancers and cardiovascular diseases in humans (1–4). Epidemiologic studies have shown variations among countries in incidences of many diseases, including cardiovascular disease, breast, endometrial, and prostate cancers. Asian men and women have a much lower incidence of breast and prostate cancer than populations from Western developed countries, but their incidences increase rapidly when they migrate to high-risk areas, suggesting that lifestyle factors are involved in disease occurrence (5). In general, Asian populations consume low-fat diets with large intakes of plant-derived estrogenic compounds known as phytoestrogens. Data on the direct relation of blood concentrations of phytoestrogens with human disease is not conclusive because of different study designs and methodologies, and, in particular, different laboratory methodology (6,7).

There are 2 main subclasses of phytoestrogens: isoflavones that occur in large amounts only in soy and soy products (such as miso and tofu) and mammalian lignans that occur as precursors in whole grains, fruit, and vegetables. The most important mammalian lignans, enterolactone and enterodiol, are formed by the gut microflora from the plant lignans matairesinol and secoisolariciresinol, respectively. Coumestans, a third type of phytoestrogen, are less studied. In Western populations, consumption of soy or soy-based products is usually low. It has therefore been hypothesized that, when studying relations with diseases in Western populations, lignans may be as important as isoflavones because of the widespread occurrence of lignan precursors in plant foods. However, little is known about the variation in plasma concentrations of isoflavones and lignans in healthy European people. Before designing and conducting costly and time-consuming, large-scale and nested, case-control studies to determine the relation between phytoestrogens and chronic disease occurrence, we wanted to find out whether concentrations vary across Europe. We expected concentrations of isoflavones to be higher in southern European countries due to use of specific herbs that may contain isoflavones (8). Also, for lignans, concentrations may be higher in the south because of a generally higher consumption of fruit and vegetables and more frequent drinking of wine (9,10).

In the European Prospective Investigation into Cancer and Nutrition (EPIC) blood samples were collected in a standardized way from 386,080 healthy subjects, which makes it one of the largest biorepositories in the world (11). Also, a vegetarian/vegan cohort was recruited by the EPIC study center in Oxford (UK), where consumption of soy and soy-based products is higher than in nonvegetarian populations (12).

We report here concentrations of 7 types of phytoestrogens that were measured in 1414 men and women from 16 geographical areas in 9 European countries. We also examined the influence of the study center location on the variation in plasma phytoestrogen concentrations.

## Subjects and Methods

*Study design.* EPIC is a multicenter prospective cohort study investigating the relation between diet, nutritional and metabolic characteristics, various lifestyle and environmental factors, and the risk of cancer, cardiovascular diseases, diabetes and other chronic diseases (13). Twenty-three research centers in 10 European countries are participating in the EPIC study. The collection of data and blood samples started in 1992 and follow-up is planned for at least 20 y.

Nutritional data of ~500,000 EPIC participants was collected through FFQ or a dietary history. In a random subsample of all participants (7%) a 24-h dietary recall was taken with the aim of calibrating nutritional data; this is known as the calibration study (14). A sample of the calibration set (~3000 participants) was selected for several studies on biomarkers (15–17). This sample was further reduced for the present study by taking a gender- and age-stratified subsample.

*Study population.* In this analysis, 16 study centers in geographical areas (regions) were designated by grouping them within the EPIC study: France (Paris and surroundings), Florence (central Italy), Varese/Turin (northern Italy), Ragusa/Naples (southern Italy), northern Spain (San Sebastian, Pamplona, Oviedo), Granada (southern Spain), Murcia (southeastern Spain), Cambridge (subjects living in Norfolk), Oxford center (vegetarians and vegans living throughout the UK), the Netherlands (including subjects from Utrecht and Bilthoven), Athens in Greece, Heidelberg (southwest Germany), Potsdam (Eastern Germany), Malmö (southern Sweden), Umeå (northern Sweden), and Denmark (including subjects from Aarhus and Copenhagen). Subsamples in each of these regions were randomly selected from the calibration sample of stored blood samples. The selection followed a stratified sampling scheme with 100 subjects: 25 men and 25 women in each of 2 age categories (50–54 or 55–60 y of age at the time of blood collection). For France, where only women were recruited, only 50 subjects were selected. Norway was not included because, at that time, the number of plasma samples collected was limited.

As far as possible, equal numbers of subjects were selected for each season at which the blood sample was collected. In total, 1550 subjects were selected to participate in the study. After excluding participants with missing 24-h recalls ($n = 21$), prevalent cancer ($n = 57$), or incident cancer (between blood collection and blood analysis, $n = 58$), a total of 1414 were left for the present analyses (718 female, 696 male).

*Blood collection.* When study participants visited the local study center for the completion of questionnaires and anthropometric measurements, a 30-mL peripheral blood sample was drawn in three 10-mL Safety Monovettes (Sarstedt) from mostly nonfasting subjects. One of the 3 syringes did not contain anticoagulant and 2 syringes contained 1 mL of 3.13% trisodium citrate as anticoagulant. Filled syringes were kept at 5–10°C, protected from light, and transferred to a central laboratory for further processing and production of aliquots. After centrifugation at $1500 \times g$ for 20 min, blood fractions (serum, plasma, buffy-coat and red-blood cells) were placed in heat-sealed 0.5-mL plastic straws, using a semiautomatic machine (CBS-IMV Technologies). Samples were initially frozen at −80°C in a horizontal position to prevent concentration gradients and then transferred into liquid nitrogen (−196°C). Further details for blood drawing and processing are described in detail elsewhere (15–17). Subjects gave informed consent at the time of data and blood collection, in accordance with local and the International Agency for Research on Cancer's Ethical Committee requirements.

*Laboratory analyses.* Aliquots of citrated plasma were extracted from the central biorepository and arranged in batches containing plasma from subjects from several centers. Samples were stored in liquid nitrogen (−196°C). At the Dunn Human Nutrition Unit, aliquots were rapidly thawed at room temperature (the large area:volume ratio of the CBS straws ensures a rapid temperature shift) and triply $^{13}C_3$-labeled standards in methanol were added to 150 μL samples. Conjugates were hydrolyzed to the aglycones, extracted on Strata C18-E SPE cartridges, and dried under nitrogen. Samples were redissolved in 40% methanol then analyzed under subcontract at HFL, using methods developed at the Dunn Human Nutrition Unit using isotope dilution liquid chromatography/tandem MS (18). Information on quality assurance and methodology are described in detail elsewhere (18). Empirically calculated limits of detection were 0.1 μg/L (0.4 nmol/L) for all types of phytoestrogens. Interassay CV in concentrations of mean population concentrations were all <10%, intra-assay variation was <6.5%. Analyses of 7 phytoestrogens were conducted: daidzein; genistein and glycitein; 2 metabolites of daidzein, O-desmethylangolensin (O-DMA) and equol; and 2 lignans, enterodiol and enterolactone.

Plasma concentrations in subjects from Sweden (Malmö and Umeå) were multiplied by 0.83 to obtain dilution concentrations for these heparinized plasma samples comparable to the citrated plasma used in the core EPIC cohorts. This dilution factor was based on the assumption

that, in general, the hematocrit was 0.45 and that, after centrifugation of the blood with anticoagulant, all citrate was in the plasma (15,16).

*Statistical methods.* The low volume (150 μL) of sample available for biochemical analyses meant that it was difficult to detect low concentrations. Correspondingly, high percentages of subjects were reported with phytoestrogen concentrations below the detection concentration of 0.1 μg/L (0.4 nmol/L): 18% of participants showed daidzein concentrations below the detection limit, 11% for genistein, 80% for glycitein, 73% for O-DMA, 62% for equol, 31% for enterodiol and 5% for enterolactone. For these persons, the phytoestrogen levels were imputed at 0.1 μg/L (0.4 nmol/L).

Because phytoestrogens showed skewed distributions, data were log-transformed. Pearson correlation coefficients were computed between the 7 phytoestrogens. Geometric means and 95% CI were presented for back-transformed data. Means were adjusted for age, BMI, sex, alcohol intake, smoking, and analytical batch number, and tested (2-sided) for differences between gender and centers using univariate ANOVA. $P < 0.05$ was considered significant.

To find out whether a "study center region," as a proxy for local dietary habits, was an important determinant for plasma phytoestrogen concentrations, we assessed the partial $R^2$ for study center region. We used multivariate regression analysis using GLM procedures in SPSS. This analysis was repeated after excluding the Oxford, UK center, to evaluate variation in nonvegan/vegetarian populations. Analyses were adjusted for nondietary factors thought to be associated with consumption of foods containing phytoestrogens, i.e., gender, age (y), BMI (kg/m$^2$), alcohol consumption (mean g/d in the year preceding study recruitment) and smoking status (nonsmoker, previous smoker, current smoker, unknown). The results were also adjusted for the analytical laboratory batch (29 batches were used). To make this feasible, plasma samples of different study center regions were distributed randomly over several batches during the laboratory measurements. Next, for the variable "batch," dummies were made and these "dummy variables" were added to the statistical models. The significance of predictors was based on type-III Sum of Squares estimates. The partial $R^2$ was calculated by dividing the sum of squares of the independent variable by the total sum of squares. Data were analyzed using the SPSS, System for Windows, version 12.0.

## Results

Age and sex stratification of the population sampling was successful: mean age was 54–55 y in all regions and the percentage of females ranged from 42 (Malmö) to 53% (Ragusa/Naples), except in France where the study included only women. Study participants in Murcia had the highest mean BMI (30 kg/m$^2$), whereas mean BMI was low for the vegetarian/vegan cohort in Oxford (23 kg/m$^2$) and in French females (24 kg/m$^2$). Of all study participants, 49% reported to have never smoked, whereas mean alcohol consumption was 15 g/d (**Table 1**).

In the total population geometric mean concentrations for all 7 phytoestrogens were <3 μg/L (12 nmol/L): enterolactone concentrations were highest [2.7 μg/L (9.0 nmol/L)], followed by genistein [1.7 μg/L (6.3 nmol/L)] and daidzein [0.8 μg/L (3.1 nmol/L)]. Geometric mean phytoestrogen concentrations did not differ between males and females, except for enterolactone, which was slightly higher for women ($P = 0.04$) (**Table 2**).

Intercorrelations between the 3 isoflavones were all high (Pearson correlation coefficients >60%), intercorrelations between daidzein and its metabolite O-DMA was high (70%), but relatively low for equol (21%). Correlation between the lignans enterodiol and enterolactone was moderate (46%), whereas correlations between enterolignans and isoflavones were all <25% (**Table 3**). Correlations between phytoestrogens and age or BMI were all low, i.e., <5%, except for enterolactone, which was moderately inversely related with BMI (−18%, $P < 0.01$).

All study centers had high percentages of subjects with O-DMA, equol, and glycitein concentrations below the detection limit (**Table 4**). Also, concentrations for enterodiol had to be interpreted with caution due to high percentages of subjects with values below the detection concentration (~30%).

When the Oxford (UK) center was excluded, daidzein concentrations varied between 0.3 μg/L (1.2 nmol/L) (south of Europe, i.e., Ragusa/Naples and Greece) to 3.9 μg/L (15.3 nmol/L) (Netherlands); genistein concentrations varied between 0.7 μg/L (2.6 nmol/L) [southern Europe (Ragusa/Naples and Greece) and Umeå (Sweden)] and 6.1 μg/L (22.6 nmol/L) (Netherlands). For enterolactone, concentrations were highest in Denmark [8.2 μg/L (27.5 nmol/L)] and lowest in northern Italy (Varese/Turin) [2.1 μg/L (7.0 nmol/L)]. For all centers, enterolactone concentrations were higher than daidzein or genistein concentrations, except for the Netherlands, Cambridge (UK), and Heidelberg (Germany).

**TABLE 1** Characteristics of the study population[1]

| Center | *n* | Age at entry | Women | BMI | Never smokers | Alcohol intake |
|---|---|---|---|---|---|---|
| | | *y* | *n (%)* | *kg/m$^2$* | *n (%)* | *g/d* |
| Umea | 96 | 54 ± 5 | 50 (52) | 25 ± 3 | 52 (54) | 9 ± 24 |
| Malmo | 77 | 55 ± 3 | 32 (42) | 26 ± 4 | 21 (27) | 14 ± 29 |
| Denmark | 100 | 54 ± 3 | 50 (50) | 26 ± 4 | 44 (44) | 23 ± 30 |
| Cambridge | 95 | 54 ± 3 | 46 (48) | 25 ± 3 | 47 (50) | 15 ± 36 |
| Netherlands | 94 | 54 ± 3 | 46 (49) | 26 ± 3 | 29 (31) | 14 ± 28 |
| Potsdam | 85 | 54 ± 3 | 41 (48) | 27 ± 3 | 40 (47) | 14 ± 21 |
| Heidelberg | 93 | 55 ± 3 | 47 (51) | 26 ± 4 | 41 (44) | 18 ± 29 |
| France | 41 | 54 ± 3 | 41 (100) | 24 ± 4 | 30 (73) | 15 ± 16 |
| Varese/Turin | 99 | 55 ± 3 | 49 (50) | 25 ± 4 | 43 (43) | 22 ± 27 |
| Florence | 96 | 55 ± 3 | 49 (51) | 26 ± 4 | 45 (47) | 18 ± 25 |
| Ragusa/Naples | 95 | 54 ± 3 | 50 (53) | 28 ± 4 | 41 (43) | 10 ± 16 |
| Northern Spain | 95 | 55 ± 3 | 48 (51) | 29 ± 4 | 57 (60) | 16 ± 25 |
| Murcia | 95 | 54 ± 3 | 48 (51) | 30 ± 4 | 54 (57) | 18 ± 26 |
| Granada | 96 | 54 ± 3 | 49 (51) | 29 ± 4 | 64 (67) | 10 ± 22 |
| Greece | 87 | 54 ± 3 | 38 (44) | 27 ± 3 | 44 (51) | 12 ± 21 |
| Oxford | 70 | 54 ± 3 | 34 (49) | 23 ± 3 | 37 (53) | 6 ± 13 |
| Total | 1414 | 54 ± 3 | 718 (51) | 27 ± 4 | 689 (49) | 15 ± 26 |

[1] Values are means ± SD or *n* (%).

**TABLE 2** Geometric mean (95% CI) and median plasma phytoestrogen concentrations of European males and females

| Gender | Daidzein | Genistein | Glycitein | O-DMA | Equol | Enterodiol | Enterolactone |
|---|---|---|---|---|---|---|---|
| Male, $n = 696$ | | | | $\mu g/L$[1] | | | |
| Mean (95% CI) | 0.89 (0.78,1.02) | 1.77 (1.55,2.02) | 0.14 (0.13,0.15) | 0.17 (0.16,0.17) | 0.15 (0.14,0.16) | 0.32 (0.29,0.35) | 2.48 (2.24,2.75) |
| Median | 0.81 | 1.77 | 0.10 | 0.10 | 0.10 | 0.25 | 3.13 |
| ND,[2] *% of subjects* | 17 | 12 | 78 | 69 | 59 | 28 | 5 |
| Female, $n = 718$ | | | | | | | |
| Mean (95% CI) | 0.80 (0.70,0.90) | 1.70 (1.51,1.92) | 0.13 (0.12,0.14) | 0.15 (0.15,0.15) | 0.15 (0.14,0.17) | 0.29 (0.27,0.32) | 2.90 (2.62,3.20)* |
| Median | 0.68 | 1.55 | 0.10 | 0.10 | 0.10 | 0.23 | 3.20 |
| ND,[2] *% of subjects* | 19 | 9 | 83 | 77 | 64 | 34 | 4 |
| Total, $n = 1414$ | | | | | | | |
| Mean (95% CI) | 0.84 (0.77,0.92) | 1.73 (1.59,1.90) | 0.13 (0.13,0.14) | 0.16 (0.15,0.16) | 0.15 (0.15,0.16) | 0.30 (0.29,0.33) | 2.68 (2.50,2.88) |
| Median | 0.73 | 1.68 | 0.10 | 0.10 | 0.10 | 0.24 | 3.15 |
| ND,[2] *% of subjects* | 18 | 11 | 80 | 73 | 62 | 31 | 5 |

* Different from males, $P < 0.05$.

[1] To convert $\mu g/L$ to nmol/L, the following conversion factors should be used: genistein: × 3.70; daidzein: × 3.93; glycitein: × 3.52; O-DMA: × 3.87; equol: × 4.13; enterolactone: × 3.35; enterodiol: × 3.31.

[2] Not detected; data for these subjects were imputed at 0.1 $\mu g/L$ (0.4 nmol/L).

Vegetarian and vegan participants of the Oxford center (UK) showed the highest concentrations of isoflavones: concentrations for daidzein [20 $\mu g/L$ (78.6 nmol/L)] and genistein [40 $\mu g/L$ (148 nmol/L)] were 5–50 times higher than those in the other study center regions. For enterolactone, concentrations in Oxford center (UK) were also high [5.3 $\mu g/L$ (17.8 nmol/L)], although not as high as in Denmark [8.2 $\mu g/L$ (27.5 nmol/L)].

Geometric mean concentrations were ranked from the north to the south, and clear gradients over Europe were not observed (Table 4). In general, plasma concentrations of all phytoestrogens were low in southern European countries, with the exception of lignans in Greece. Excluding Oxford (UK), concentrations of daidzein and genistein varied 13-fold and 8-fold, respectively (Table 4). For lignans, variation was less: 4-fold for enterolactone. Concentrations of all phytoestrogens differed among the various centers (ANOVA, $P < 0.005$).

The total explained variance for models, including all 16 study centers and the characteristics of age, gender, BMI, smoking, alcohol intake, and laboratory batch (3–36%) was generally higher than that for the same models excluding the Oxford study center (1–17%). In all models, with or without the Oxford center, the study center region was the most important determinant of the phytoestrogen concentrations and was significant for all 7 (**Table 5**).

## Discussion

This study showed that, when compared with Asian populations, plasma concentrations of phytoestrogens in Europeans are generally low(19,20) but that there still is substantial variation. The vegetarian/vegan cohort of the Oxford (UK) study center region showed 5–50 times higher isoflavones concentrations than in the other European centers. This was most likely caused by the regular consumption of soy-based products by vegetarians/vegans to replace meat products (12). A study in the EPIC Oxford (UK) participants clearly showed a relation between the intake of soy foods, especially soy milk, and blood concentrations of daidzein and genistein (21). Although they were high compared with nonvegetarian European populations, mean concentrations of diadzein and genistein in Oxford, UK, were still 60–70% lower than concentrations in Japanese people (19,20). Concentrations in the upper quartile were close to concentrations in Asian populations where consumption of soy traditionally is high (21).

Although low, isoflavones concentrations still varied 8-fold (genistein) to 13-fold (daidzein) in nonvegetarian European populations. Contrary to our expectations, concentrations were low in centers of southern Europe (Greece, Spain, Italy, and France); intermediate in centers in northern countries (Sweden, Denmark); and relatively high in mid-European countries (UK, Germany, and The Netherlands). We believe that this is explained by a more frequent consumption of processed and industrial products that contain soy additives in mid-European countries, i.e., bread, bakery products, and processed meats (22). In the EPIC Norfolk (UK) participants, 60% of isoflavones were consumed as bread and bakery products (23). A different use of phytoestrogen-containing supplements across Europe may also explain the observed geographical differences in concentrations, but we did not have information on this point. If this was caused by the use of isoflavones for relief of menopausal complaints, we would expect differences between men and women in the

**TABLE 3** Pearson correlation coefficients between 7 phytoestrogens in 1414 European adults[1]

| | Daidzein | Genistein | Glycitein | O-DMA | Equol | Enterodiol | Enterolactone |
|---|---|---|---|---|---|---|---|
| Daidzein | 1.00 | 0.81 | 0.70 | 0.70 | 0.21 | 0.24 | 0.21 |
| Genistein | | 1.00 | 0.63 | 0.56 | 0.21 | 0.22 | 0.12 |
| Glycitein | | | 1.00 | 0.72 | 0.21 | 0.23 | 0.11 |
| O-DMA | | | | 1.00 | 0.26 | 0.25 | 0.22 |
| Equol | | | | | 1.00 | 0.17 | 0.19 |
| Enterodiol | | | | | | 1.00 | 0.46 |
| Enterolactone | | | | | | | 1.00 |

[1] All correlations were significant, $P < 0.01$.

**TABLE 4** Adjusted geometric means (95% CI) of 7 plasma phytoestrogen concentrations in 1414 European adults[1]

| | Geometric mean (95% CI), ND,[2] *% of subjects* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Daidzein | Genistein | Glycitein | O-DMA | Equol | Enterodiol | Enterolactone |
| Umea | 0.5 (0.3,0.8) 23 | 0.7 (0.4,1.2) 19 | 0.1 (0.1,0.2) 87 | 0.1 (0.1,0.2) 82 | 0.2 (0.1,0.3) 45 | 0.2 (0.1,0.3) 40 | 4.5 (2.8,7.1) 0 |
| Malmo | 0.5 (0.3,0.9) 13 | 1.1 (0.6,1.8) 4 | 0.1 (0.1,0.2) 91 | 0.1 (0.1,0.2) 82 | 0.2 (0.1,0.3) 47 | 0.2 (0.1,0.3) 42 | 3.0 (1.9,4.8) 3 |
| Denmark | 1.1 (0.6,1.9) 8 | 1.2 (0.7,2.2) 16 | 0.1 (0.1,0.2) 88 | 0.2 (0.1,0.2) 61 | 0.1 (0.1,0.2) 75 | 0.5 (0.3,0.8) 19 | 8.2 (5.0,13.7) 4 |
| Cambridge | 2.1 (1.6,2.9) 6 | 4.2 (3.0,5.7) 2 | 0.2 (0.1,0.2) 60 | 0.2 (0.1,0.2) 57 | 0.1 (0.1,0.2) 65 | 0.3 (0.2,0.3) 31 | 2.6 (1.9,3.4) 6 |
| Netherlands | 3.9 (2.9,5.4) 2 | 6.1 (4.4,8.4) 1 | 0.2 (0.1,0.2) 47 | 0.3 (0.2,0.3) 41 | 0.2 (0.1,0.2) 68 | 0.2 (0.2,0.3) 46 | 2.8 (2.1,3.7) 5 |
| Potsdam | 0.9 (0.7,1.2) 13 | 1.6 (1.2,2.3) 6 | 0.1 (0.1,0.2) 80 | 0.1 (0.1,0.2) 74 | 0.2 (0.1,0.2) 60 | 0.3 (0.2,0.4) 32 | 3.1 (2.3,4.1) 2 |
| Heidelberg | 1.0 (0.8,1.4) 11 | 2.8 (2.0,3.8) 4 | 0.1 (0.1,0.2) 80 | 0.1 (0.1,0.2) 80 | 0.2 (0.1,0.2) 57 | 0.3 (0.3,0.4) 29 | 2.7 (2.1,3.7) 5 |
| France | 0.7 (0.4,1.1) 17 | 1.8 (1.1,2.9) 2 | 0.1 (0.1,0.2) 83 | 0.1 (0.1,0.2) 80 | 0.2 (0.1,0.2) 61 | 0.3 (0.2,0.5) 34 | 3.4 (2.2,5.2) 2 |
| Varese/Turin | 0.6 (0.3,0.8) 23 | 1.1 (0.8,1.5) 18 | 0.1 (0.1,0.2) 86 | 0.2 (0.1,0.2) 79 | 0.2 (0.1,0.2) 59 | 0.3 (0.2,0.4) 31 | 2.1 (1.6,2.7) 8 |
| Florence | 0.4 (0.3,0.6) 34 | 1.1 (0.8,1.5) 13 | 0.1 (0.1,0.2) 93 | 0.1 (0.1,0.2) 82 | 0.2 (0.1,0.2) 57 | 0.3 (0.2,0.4) 24 | 2.7 (2.1,3.6) 5 |
| Ragusa/Naples | 0.3 (0.2,0.5) 40 | 0.7 (0.5,1.0) 22 | 0.1 (0.1,0.2) 88 | 0.1 (0.1,0.2) 85 | 0.2 (0.1,0.2) 62 | 0.3 (0.2,0.3) 31 | 2.4 (1.8,3.2) 6 |
| Spain North | 0.5 (0.3,0.6) 17 | 1.1 (0.8,1.4) 12 | 0.1 (0.1,0.2) 91 | 0.1 (0.1,0.2) 77 | 0.1 (0.1,0.3) 61 | 0.2 (0.1,0.2) 42 | 2.3 (1.7,3.0) 6 |
| Murcia | 0.5 (0.3,0.6) 18 | 1.2 (0.9,1.7) 11 | 0.1 (0.1,0.2) 93 | 0.1 (0.1,0.2) 87 | 0.1 (0.1,0.2) 66 | 0.3 (0.2,0.4) 26 | 3.2 (2.4,4.3) 6 |
| Granada | 0.6 (0.4,0.8) 10 | 1.3 (1.0,1.8) 13 | 0.1 (0.1,0.2) 90 | 0.1 (0.1,0.2) 90 | 0.1 (0.1,0.2) 59 | 0.2 (0.2,0.3) 34 | 2.3 (1.8,3.1) 5 |
| Greece | 0.3 (0.2,0.4) 40 | 0.7 (0.5,1.0) 16 | 0.1 (0.1,0.2) 97 | 0.1 (0.1,0.2) 91 | 0.1 (0.1,0.2) 68 | 0.4 (0.3,0.5) 26 | 4.5 (3.3,6.0) 2 |
| Total[3] | 0.7 (0.6,0.9) 18 | 1.5 (1.4,1.6) 11 | 0.1 (0.1,0.1) 80 | 0.1 (0.1,0.1) 73 | 0.1 (0.1,0.1) 62 | 0.3 (0.3,0.3) 31 | 2.6 (2.4,2.8) 5 |
| Oxford | 20 (14,29) 6 | 40 (27, 109) 1 | 0.9 (0.8,1.1) 19 | 2.1 (1.7,2.6) 8 | 0.3 (0.2,0.4) 77 | 1.1 (0.8,1.5) 10 | 5.3 (3.8,7.5) 6 |

[1] Data were adjusted for age (mean: 55 y), sex (women), smoking (never smokers), BMI (mean: 26), alcohol intake (mean in women 9 g/d), and Batch.
[2] Not detected; data for these subjects were imputed at 0.1 $\mu$g/L (0.4 nmol/L).
[3] Excludes Oxford.

geographical distributions, but this was not the case. Concentrations for isoflavones were similar for men and women. This is in accordance with other studies of British and Japanese populations (19,20).

Equol has been proposed as the important metabolite of daidzein that is found in vitro to have the highest estrogenic capacity (24). For equol, large percentages of participants showed very low levels (below detection limit). Also, for Oxford (UK), 77% of participants showed low values despite a relatively high consumption of daidzein (12,21). However, 16% of the Oxford study population showed concentrations of equol >4.8 $\mu$g/L (20 nmol/L), although this was only 0–2% in the other nonvegetarian EPIC populations. In another study in the UK, 0% of men and 2% of women had equol concentrations >20 nmol/L, whereas in a Japanese population this was 40–60% (20). Challenging people by providing them with high amounts of daidzein (or regular soy consumption) during several days will stimulate the metabolism from daidzein to equol (24). However, even after challenging, there is a proportion of people not able to produce equol, most likely because of the absence of a specific bacterial gut flora. Also, the use of antibiotics has been shown to temporarily reduce the ability to produce equol. Information about antibiotic use was not available for our study participants. Several studies in Western men and women showed that after challenging them with soy, 60–70% are able to metabolize daidzein to equol (25,26).

The variation of European enterolactone concentrations was less than for isoflavones. Like isoflavones, we expected the highest enterolactone concentrations in southern-European countries, due to a higher consumption of fruit and vegetables in these areas (9). However, the highest concentrations of enterolactone were in Denmark, and there were relatively high concentrations in the vegetarian/vegan cohort of Oxford (UK), in centers in Greece, and in France. Concentrations were slightly lower than those

**TABLE 5** Percentage of partial and total explained variance ($R^2$) for 7 phytoestrogens concentrations by models including the characteristics center/region, age, sex, BMI, smoking, alcohol, and batch[1]

| | Daidzein | Genistein | Glycitein | O-DMA | Equol | Enterodiol | Enterolactone |
|---|---|---|---|---|---|---|---|
| | | | | % | | | |
| Study center region, $n = 16$ | 30[a] | 26[a] | 32[a] | 33[a] | 3[a] | 11[a] | 4[a] |
| Age | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | 0.3[a] | 0.3[a] |
| Sex | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | <0.1 | <0.2 |
| BMI | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | <0.1 | 2.0[b] |
| Smoke | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | <0.1 | 0.9[b] |
| Alcohol | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | <0.1 | <0.2 |
| Batch | <0.2 | <0.1 | <0.6 | <0.3 | <0.1 | <0.1 | <0.2 |
| Total | 32 | 28 | 36 | 36 | 3 | 11 | 8 |

[1] $n = 29$.
[a] $P < 0.01$.
[b] $P < 0.05$.

reported in Japanese populations (20). Lignans occur at high concentrations in tea, berries, flaxseed, nuts, and whole grains, and the observed European variation most likely reflects consumption of these products (27,28). However, precursors of lignans are consumed from plant foods and the good condition of intestinal flora is crucial for the production of mammalian lignans. In theory, regional differences in lignan concentrations are not only determined by regional differences in consumption of above foods, but also by regional differences in the condition of the intestinal flora, which can be affected by the use, for e.g., of antibiotics or foods (probiotics) or diseases that affect the gut flora. We did not have information about these factors.

Despite the apparently low circulating concentrations of phytoestrogens in plasma compared with populations consuming soy, concentrations are 500–1000 times those of circulating estradiol in postmenopausal women. In EPIC Norfolk, these concentrations were sufficient to interact with gene variants in affecting plasma estradiol concentrations, to affect concentrations of SHBG (29,30). Nevertheless, studies, including dietary intervention studies where exposure to phytoestrogens was high, were not consistent with respect to phytoestrogens and plasma estradiol and SHBG concentrations [see references in (30)]. Also, effects on breast cancer risk were not consistent (23,31). The biological activity of phytoestrogens is complex. Once absorbed, phytoestrogens are extensively reconjugated, and isoflavones and lignans are present in the circulation in predominantly conjugated forms (32). The biological activity of these conjugated forms seems less clearly understood than that of unconjugated forms, but it does seem that they may be generally less potent in terms of their binding with the estrogen receptor and stimulating transcription (33).

For this study, only 150 $\mu$L of plasma was available. Most likely this caused the high percentages of concentrations below the detection level for glycetein, O-DMA, and equol (80, 73, and 62%, respectively). Characteristics of these study participants were similar to those with detectable levels (data not shown). However, region-specific mean levels may have been overestimated by including participants with levels considered to be at the detection level. Nevertheless, mean levels that we report here are similar to levels previously reported for European populations (20).

This study is the largest existing study showing plasma phytoestrogen concentrations in healthy European people. Methods of blood collection, sample handling, and storage were highly standardized in the 16 study centers. Laboratory assays were done in a central laboratory that was experienced in measuring phytoestrogens in blood, urine, and foods. In most participants nonfasting blood samples were stored. The time of the blood sampling was recorded (except in Sweden). Adjustment for blood sampling time, as a proxy for fasting status, did not change the results. Due to the stratified selection of the sample, potential age and gender effects were taken into account. In addition, information on BMI, batch, drinking of alcohol, and smoking was available to evaluate the relative importance of study center location to predict levels of blood phytoestrogen concentrations. For all types of phytoestrogens, study center region was the most important and significant predictor of plasma phytoestrogen concentrations in Europe. Most likely, local and regional nutritional habits are the underlying explanation for this observation.

In conclusion, this study showed that variation in concentrations of phytoestrogens is present in European populations. We now have the ability to investigate interactions among gene variants, hormones, and plasma phytoestrogen concentrations using stored samples in EPIC in relation to the large numbers of breast and other cancers.

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