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# Urinary 2/16 estrogen metabolite ratio levels in healthy women: a review of the literature

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## Abstract

This is a summary of the published literature on the urinary 2/16 estrogen metabolite ratio in human populations, and a report the observed range of normal values in healthy women. Original research studies that included the measurement of urinary estrogen metabolites in human subjects were identified through an extensive Medline search; 43 distinct studies were indentified, including a total of 6802 healthy women. The range of mean values of the 2/16 ratio measured with the ELISA method varied from 0.98 to 1.74; in studies of pre-menopausal women the range of mean values was 1.5 to 2.74, in studies of post-menopausal women mean values ranged from 1.15 to 2.25. The heterogeneity across studies was highly significant (p-value Q test: <0.0001). In multivariable analyses, only race confirmed its role as an independent predictor of 2/16 ratio (F value: 7.95; p value: 0.009), after adjustment for age and menopausal status. There appears to be a large body of data on the 2/16 urinary ratio in healthy women. However, summary estimates are difficult to perform due to the high variability of the published study-specific values. The data suggests that race may be a contributor to 2/16 urinary ratio levels.

## Introduction

Estrogen metabolism is a complex process that starts with a reversible chemical reaction, the conversion of estradiol to estrone in the C17 position. After this step, estrone undergoes hydroxylation at positions C2, C4 or C16 [1, 2], with the production of several metabolites, including 2-hydroxyestrone (2-$OHE_1$), 4-hydroxyestrone (4-OHE), 16-alpha-hydroxyestrone (16α-$OHE_1$) or estriol (1); the 2-hydroxylation represents the prevalent metabolic pathway and takes place mostly in the liver [3]. Both the 2-$OHE_1$ and the 16α-$OHE_1$ metabolites have estrogenic properties, but with different activity; the 16α-$OHE_1$ metabolite binds to the estrogen receptor and has similar properties to those of estradiol and thus, has been classified as an estrogen-like compound. The 2-$OHE_1$ metabolite has nearly no affinity to the estrogen receptor; therefore it is virtually devoid of estrogen activity. Furthermore, the metabolic pathway produces either the 2-$OHE_1$ or 16α-$OHE_1$ in a mutually exclusive way



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[1], thus their ratio may be a useful measure of a woman's exposure to estrogen-like metabolites.

Some constitutional factors such as genetic polymorphisms and family history of breast cancer are associated with the levels of the 2/16 ratio measured in urine [4]. Environmental and behavioral factors, including diet, physical activity, body weight, hormone therapy, smoking and alcohol consumption have been examined as potential modifiers of the 2/16 ratio. Exercise may increase 2-$OHE_1$ production [5], while higher body weight may favor the 16 pathway [6]. Similarly, a diet rich in cruciferous vegetables is suggested to modify the 2/16 ratio by increasing the 2-hydroxylation pathway, as previously reviewed [7]. Hormone replacement therapy seems to increase 2-$OHE_1$ production more than 16α-$OHE_1$ [8].

It has been hypothesized that estrogen metabolites are mutagens through the production of depurinating DNA adducts [9].

Urinary estrogen metabolites have been also studied in relation to breast [studies summarized in [10], endometrial [11] and prostate cancer [12]. However, prior to establishing associations between the metabolites and cancer, it is crucial to assess the range of the urinary estrogen metabolite levels in healthy women. Although several studies have reported on this topic, the expected range of the urinary 2/16 ratio among healthy women remains unknown. Aspects that also need to be addressed are the variation of the 2/16 ratio in healthy women with regards to ethnicity, menstrual cycle, and menopausal status, as well as individual characteristics, such as age and lifestyle factors. Additional variability could be introduced by the sensitivity/specificity of the laboratory kit used for each metabolite, as well as variability across laboratory kits.

This review aims at summarizing the published literature on the urinary 2/16 estrogen metabolite ratio in human populations, with the objective of reporting the observed range of normal values in healthy women.

## Materials and Methods

### Study Identification and Selection Criteria

Origin al research studies that included the measurement of urinary estrogen metabolites in human subjects were identified by searching the National Library of Medicine and National Institutes of Health Pubmed database. The search strategy involved the following keyword search terms: estrogen metabolites, 2-$OHE_1$, 16α-$OHE_1$, 2-hydroxyestrone, 16α-hydroxyestrone, hormone metabolites; additional limits were females and English language. This search is current as of December 1, 2009. Each of the 1,854 citations and abstracts were reviewed, and articles were considered eligible for inclusion if they met the following pre-determined inclusion criteria: (1) an original research study, (2) inclusion of healthy women, (3) inclusion of the ratio of 2-hydroxyestrone (2-$OHE_1$) over 16-alpha-hydroxyestrone (16α-$OHE_1$), and (4) urine as the sample source. A total of 87 articles were identified as potentially containing the measurement of the 2/16 urinary ratio in healthy women; after reviewing the details of the publications, thirty-two articles were further excluded for the following reasons: (1) methodological articles including 1 or 2 subjects [13-15], (2) did not provide the 2/16 ratio, only the individual metabolites [16-33]; in this case, a possible option would have been to calculate the ratio using the average 2/average 16, but this would have not taken into account the sample variance of the measurement, (3) in vitro studies [34, 35], (4) the ratio was calculated but not reported [36-38], (5) the metabolites were measured in plasma or serum [39-43] and (6) the population was treated with HRT [44]. Reference lists

from retrieved articles were also reviewed in order to identify additional eligible articles; no additional studies were identified.

The number of articles that met the criteria for inclusion was 56; however, study populations from 13 publications partially overlapped with each other and thus, the final number of distinct studies was 43.

### Criteria for inclusion of data from each study

If the parent study was a case-control study, only information on controls were extracted; if it was a randomized clinical trial or an intervention study, only baseline information of non-treated women were used. Follow-up measurements were not included. Some of the included studies reported on controls with and without hormone therapy or oral contraceptive intake; in this case, only data on women without treatment were included.

### Laboratory methods

Earlier studies utilized the gas chromatography-mass spectrometry technique [45], a labor intensive method [46]. In 1994, Klug et al. developed an Enzyme-Linked Immunosorbent Assay (ELISA) technique [47], which was subsequently validated in its modified version against gas chromatography-mass spectrometry [48]. The original ELISA kit did not have the appropriate limit of detection when used to measure 2-$OHE_1$ and 16α-$OHE_1$ in urine samples from postmenopausal women [14]; a modified version of the kit was developed with an increased sensitivity level of 0.625 ng/ml [49] and adjustments applied to the antibody concentrations, enzyme concentrations and standards.

### Data extraction and tabulation

For each eligible study the following information was extracted and tabulated: the main objective of the study, the number of women included, the type of laboratory method used to measure the estrogen metabolites, the number of samples collected per study subject, the time of the day/menstrual cycle (if available), method used to report the ratio (mean, median, range), and the main analyses reported in the original published paper.

### Statistical Analyses

Comparisons across studies were performed based on mean values. For studies where the median and the full range were reported, the mean was calculated according to Hozo et al. as follows: $[a+2m+b]/4+[a-2m+b]/4n$, where $m$ is the median, $a$ and $b$ are the extremes of the range, $n$ is the sample size. For studies including SD of the mean, SE was calculated as $SE=SD/\sqrt{n}$ [50]. When 95% Confidence Intervals were presented, they were used as an approximation of the SD. For studies where the range of values were reported, the variance was calculated as $1/12[(a-2m+b)^2/4+(a-b)^2]$ for sample sizes between 1 and 15, as range/4 for sample sizes between 16 and 70, as range/6 for sample sizes greater than 70. Studies were grouped based on the method of determining hormone levels, either ELISA or GC-MS studies. An attempt to summarize the evidence was performed separately for each of these groups. Before calculating summary estimates in each group, the Q test for heterogeneity [51] was performed to assess the degree of heterogeneity across studies. In the case of statistically significant heterogeneity, summary estimates were not calculated.

For the studies using the ELISA method, univariate analyses were performed to compare the 2/16 ratio in studies conducted on pre-, post- or both pre- and postmenopausal women. Additional comparisons were performed on the 2/16 ratio according to race and type of urine collection (spot, morning or 12 or more hours). The non-parametric Kruskal-Wallis test was used for these statistical comparisons of means. Linear regression analysis was applied to identify possible predictors of the ratio, after the assumption of normality was

checked. In this model, the dependent variable was the 2/16 ratio, the independent variables were categorical, and included menopausal status (classified as studies on premenopausal women, on postmenopausal women, on both pre and post menopausal women), race (classified as: White, Asian, Black, multiracial or Hispanic, unknown) and type of sample collection (classified as: spot urine, morning urine, 12 hours or more urine collection).

## Results

### Description of the studies

The 43 distinct studies included a total of 6802 healthy women (Table 1). The vast majority of the data sets (36/43) measured the 2/16 ratio using the ELISA method [4-6, 49, 52-95] while 6 studies used GC-MS [96-103], and 1 study [104] did not report the laboratory method. Self-defined ethnicity was reported in 30/43 data sets. Of these, 14 [6, 53, 59-63, 104, 65, 66, 82, 83, 86-89, 93-97] studies reported results on the 2/16 ratio separately for white women, 7 [5, 60, 64, 68, 76, 87, 90, 91, 99] reported separately for Asian women, and 4 [6, 60, 87-89, 104] for African Americans. The remaining studies included multiracial populations for which no breakdown of the results according to race was reported. Thirteen studies involved exclusively pre-menopausal women [58-62, 67, 71, 73, 85, 92-94, 96, 100-102], 15 included only post menopausal women [52, 53, 56, 57, 65, 66, 72, 74, 75, 80-84, 86, 90, 91, 98, 99, 103], and the remaining 15 included a mixture of both pre and post menopausal women, of which 6 studies presented the results stratified by menopausal status.

The number of urine measurements performed varied greatly across studies, from 1 to 8 or more. The methods for urine collection always involved the addition of ascorbic acid for preservation purposes, but the time of urine collection varied: a spot urine collection was used in 10 studies [4, 49, 52, 56, 57, 69, 70, 75, 77, 85, 95], while 18 studies [5, 6, 54, 55, 58-64, 68, 74, 76, 78-81, 87-94, 99] reported on overnight urine collection. The remaining studies included 12, 24, 48 or 72 urine collection protocols. The information was not available for one study [86].

The time of the menstrual cycle during which urine was collected from premenopausal women was also variable: while the majority of the studies collected urine regardless of time of the cycle, 13 of the publications reported collecting urine at a specific time during the menstrual cycle, or kept record of the day of the cycle when urine was collected, in order to adjust for it in the analysis [6, 49, 58-64, 67, 73, 87, 92-94, 96, 97]. The statistical approach to data presentation also varied: means were reported by most of the publications (32/43), geometric means by 8 studies [54-58, 64, 84, 90-92, 96, 97], medians by 3 studies [60, 77, 95].

### Summary estimates

An attempt to summarize the evidence was performed separately for the 36 ELISA studies and the 6 GC-MS studies. Twenty eight out of the 36 studies using ELISA presented the data as means, 7 as geometric means; 5 of the studies using GC-MS presented the results as means. A visual description of the means and SE for each study in the group using ELISA as laboratory method is presented in figure 1. The range of mean values of the 2/16 ratio varied from 0.98 to 1.74; in studies of pre-menopausal and postmenopausal women, the mean values ranged from 1.50 to 2.74 and 1.15 to 2.25, respectively. The heterogeneity across studies was highly significant (p-value Q test: <0.0001), therefore a summary estimate was not calculated.

Few variables available from the published papers could be analyzed as predictors of the 2/16 ratio. Stratified analyses by menopausal status, race and type of urine collection still showed statistically significant heterogeneity; therefore, summary estimates were not

calculated. Menopausal status was not associated with the 2/16 urinary ratio (Kruskal-Wallis p-value=0.9) (figure 2); studies that collected urine over a period of 12 or more hours consistently reported higher 2/16 ratios than studies collecting a morning or a spot sample (Kruskal-Wallis p-value=0.006) (figure 3). When race was considered, White women showed significantly higher mean values of 2/16 metabolite ratios than Asians, African Americans or mixed populations (Kruskal-Wallis p-value=0.05) (figure 4). In multivariable analyses that used the 2/16 ratio as dependent variable, only race confirmed its role as an independent predictor of 2/16 ratio (F value: 7.95; p value: 0.009), after adjustment for age and menopausal status.

### Other co-factors

Some of the studies included in Table 1 reported on cofactors that could potentially modify the 2/16 ratio. More specifically, studies which assessed the role of dietary factors on estrogen metabolite levels included the examination of flaxseed consumption [67, 75, 6], brassica vegetable consumption [65], fat and fibers intake [67], soya diet [58, 71, 73, 84, 101-103], dietary fibers [96, 97], low fat/low calories diet [85, 93] and indole-3-carbinol supplementation [27]. Almost all these studies suggest that a low calorie/low fat diet as well as the intake of cruciferous vegetables, indole-3-carbinole, flax and soy, increase 2-hydroxyestrone levels and therefore favorably modify the 2/16 ratio. Two publications did not show any differences in the 2/16 ratio with soy intake [73] or with a low fat diet [85]. Additional studies have evaluated the effects of lifestyle factors such as physical activity on estrogen metabolite levels [5, 6, 56, 59, 61, 85, 93, 100]. While moderate exercise seems not to influence the 2/16 ratio [56, 85], vigorous exercise, especially if associated with a low BMI [5, 6] and an increase in lean body mass [61, 100] may modify the ratio in a favorable way.

One study addressed the role of family history of breast cancer [92] on the 2/16 levels and reported no association; a recent investigation restricted to women with a positive family history of breast cancer [4] suggests that metabolic gene polymorphisms may be responsible for differences in 2/16 ratio within this group. Out of the studies that addressed postmenopausal hormone usage, three did not report a significant impact on the 2/16 ratio [52, 78, 79] while another study observed significant changes in the ratio in smokers only [98].

Smoking was analyzed in very few studies [98, 69, 60]; current smoking was associated with a slightly higher 2/16 ratio than non smokers in two studies [98, 60], but not in another [69].

## DISCUSSION

This study presents a review of the literature on urinary estrogen metabolite levels in healthy women. Several findings emerge from this analysis: over the years, more than 6,000 healthy women underwent urinary 2/16 ratio measurements as part of epidemiologic studies on cancer etiology or potential determinants of estrogen metabolism, including dietary and behavioral factors. Despite the availability of the 2/16 ratio on such a large population of women, and the fact that only two main laboratory methods are in use by the investigators, several methodological differences in study design, sample collection and data analysis are present. This greatly limits the possibility of conducting a combined analysis to determine the average urinary values in the healthy population of women as a whole and according to menopausal status and race. A separate analysis of studies which used the ELISA method shows that some differences in average 2/16 levels may exist according to race, with lower levels of the urinary 2/16 ratio observed in Asian and African-American populations in comparison to White women. This difference seems to hold after adjustment for type of

sample collection and menopausal status. Out of the individual studies that have addressed and evaluated potential racial differences in estrogen metabolites [6, 60, 87-89, 104], few reported data separately for Black women, and only one [60] did not confirm the result observed in the present meta-analysis.

Because of the limitations of any published data, a comprehensive re-analysis of cofactors that could explain the observed differences in 2/16 urinary levels with race was not possible. Individual studies suggest that dietary components, obesity, physical activity, smoking and a combination of all these factors are possible determinants of the 2/16 ratio in healthy women. A summary estimate of these factors could not be performed due to methodological differences across studies.

The data presented here refer to the 2/16 ratio measured in urine; most of the studies have focused on the measurement of these metabolites in urine since the 2-$OHE_1$ and 16α-$OHE_1$ metabolites are present in low concentrations in the blood. With the introduction of new technologies involving mass spectrometry, studies on serum and plasma levels will become more common. Information on the correspondence between urinary and plasma measurements is very limited, as well as between urine and breast tissue [60].

The studies presented in this review relied mostly on 1 measurement; studies with a follow-up after intervention reported a subsequent measure. Very few studies addressed within-person variability and the stability of estrogen metabolite levels over time. Chen et al. conducted a study to assess the within-person variability of the ratios of urinary 2-$OHE_1$ to 16α-$OHE_1$ [63] over a two-month period; the 2-$OHE_1$ and 16α-$OHE_1$ metabolites measured at any one time point correlated with the average ratio over the eight week study period (mean correlation coefficient: 0.85). Findings from a longitudinal study of five samples [105] collected over a year (n=34), suggests that a single measure of the estrogen metabolites may not have the ability to adequately account for at least 50% of the true variance. Therefore, questions still remain on the within-person variation of estrogen metabolites over time, the validity of a single measure over multiple measures as well as additional methodological issues concerning the measurement of estrogen metabolites.

Another aspect that needs to be addressed is the time of the menstrual cycle when the measure should be performed in pre-menopausal women. Currently, investigators have chosen to collect urine with different strategies including the following: sample collection only in a certain phase of the cycle, performing the measures two or more times in a cycle, or only recording the time of the menstrual cycle in which urine was collected and then adjusting for this factor in the analysis. Although the 2/16 OHE ratio is reported to be reproducible during the day and during the menstrual cycle [93, 94], a more uniform and standardized criterion in sample collection would likely help reduce variability and increase precision of the measurement. Ongoing collaborative projects aimed at standardization and improvement of steroid hormone measurements should be extended to include estrogen metabolism studies [106].

In addition to variability in the laboratory methods, variations across the ELISA kits used by the various investigators could also contribute to the observed heterogeneity across studies. A recent study comparing the ELISA method to both the radioimmunoassay and the mass spectrometry methods indicates that the ELISA method has higher coefficient of variation (< 14.2%) than the spectrometry method (< 9.4%) [107].

In the present review, the large heterogeneity across studies could not be explained by the available information on study design, sample collection, sample storage and laboratory methods; this may have implications for future pooling of data to address a possible association between estrogen metabolites and cancer. As previously suggested, study-

specific quantiles or percentage increases of biomarkers values in relation to cancer risk may have to be employed [108].

In conclusion, there appears to be a large body of data on the 2/16 urinary ratio in healthy women. However, summary estimates are difficult to perform due to the high variability of the data published. This review suggests that race may be a contributor to 2/16 urinary ratio levels, however this hypothesis could not be studied in more depth because of the large variability of the available data, and the lack of available information on covariates that could affect the ratio and be associated with race.

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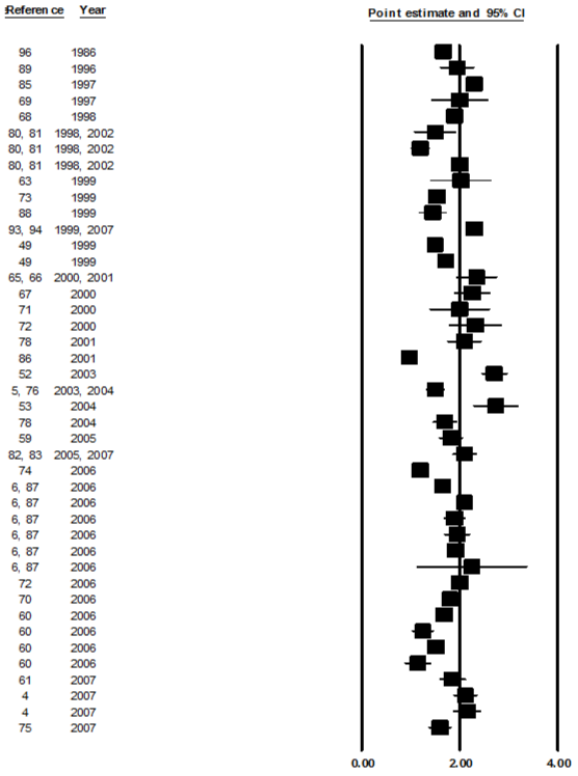


**Figure 1. Distribution of 2/16 ratio in studies where the ELISA was used. Data are presented as Mean ± SE**

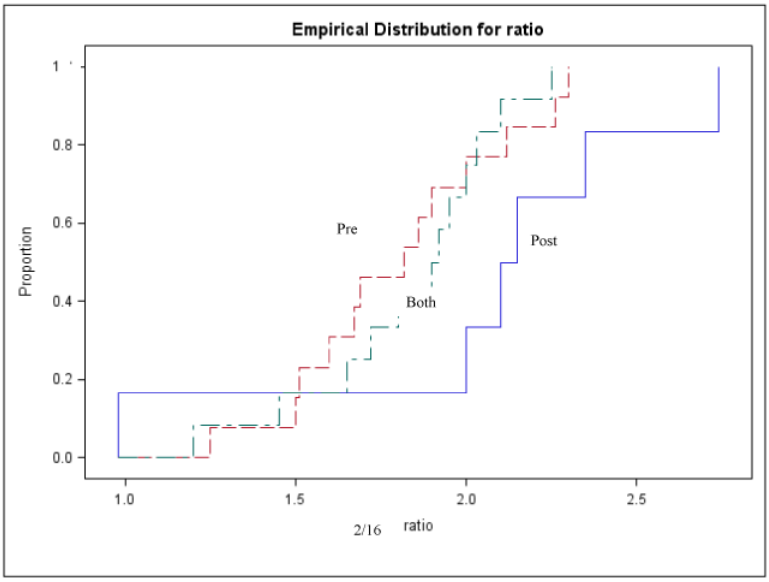


**Figure 2. Distribution of 2/16 mean values in healthy women according to menopausal status**
Kruskal-Wallis p value: 0.9

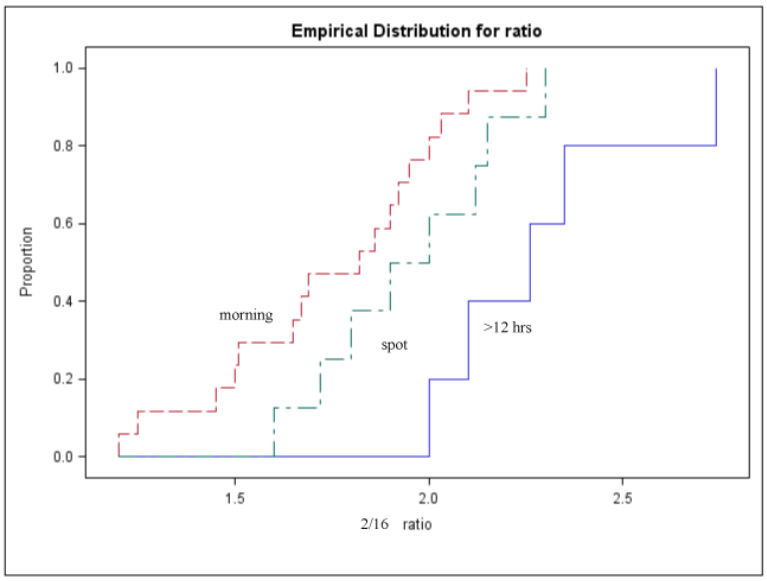


**Figure 3. Distribution of 2/16 mean values in healthy women according to type of urine collection**
Kruskal-Wallis p value: 0.006

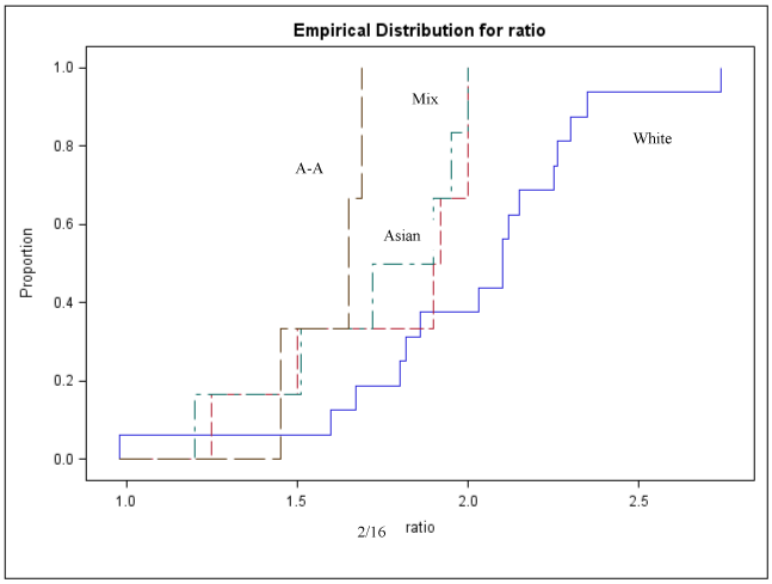


**Figure 4. Distribution of 2/16 mean values in healthy women according race**
Kruskal-Wallis p value: 0.05

**Table 1**

**Studies of the Urinary 2/16 Estrogen Metabolite Ratio in Healthy Populations of Women**

| Study reference | Main Topic of the study | N£ | Race | Age (years) | Menopausal Status | Assay | # Measures; methods of urine collection | 2/16 ratio | Statistics presented | Main analyses in the original paper |
|---|---|---|---|---|---|---|---|---|---|---|
| 96, 97^ | Dietary fiber/multiethnic | 23 | White | 31.7/34.6 | Pre | GC-MS | 2; 72 hrs, mid follicular. | 2.0 | GM, SE | Diet groups |
| 52 | HRT | 34 | N/A | N/A | Post | ELISA | 1; spot | 2.71 ± 0.84 | Mean, SD | HRT & overall |
| 53 | HRT | 174 | White | Median: 60 | Post | ELISA | 1; 24 hrs | 2.74 ± 0.24 | Mean, SE | HRT |
| 54, 55 | Equol excretion | 61 | White/Asian/AA | 25-59 | Pre, Post | ELISA | 1; overnight | 1.45 (0.4-5.6) | GM, range | Overall |
| 56, 57 | Physical activity intervention | 173 | 86% White | 50-75 | Post | ELISA | 3; spot | 1.15 (1.03-1.29) | GM, range | Baseline, after intervention |
| 58 | Soy intervention | 187 | Mostly whites | Median: 42 | Pre | ELISA | 1; morning, day 5-9 of cycle | 1.42 (1.3-1.6) | GM, 95% CI | Baseline,after soy load |
| 59 | Physical activity | 77 | White | 18-51 | Pre | ELISA | 1; first morning | 1.82 ± 0.13 | Mean, SE | Overall |
| 98 | Smoking, HRT treatment | 16 | N/A | N/A | Post | GS MS | 2; 24 hrs | NS: 1.23 ± 0.32; SMK:1.62 ±0.45 | Mean, SD | Smoking |
| 60 | Methodology on volunteers | 511 | Multiracial | 17-35 | Pre | ELISA | 1; morning., Phase of cycle recorded | White: 1.67 AA: 1.69 Asian: 1.25 Indian: 1.51 | Median, interquartile range+ | urine versus plasma |
| 49 | Assay validation | 214 | N/A | N/A | Pre, Post | ELISA | Several, morning spot follicular phase | Pre: 2.3±0.6 Post: 1.5± 0.4 | Mean, SD | Menopausal |
| 61, 62 | Aerobic fitness | 30& | White | 20-42 | Pre | ELISA | 2; first morning, luteal | 1.86$ | Mean, 95% CI | Fitness, Menstrual cycle |
| 63 | Methods | 10 | White | 23-58 | Pre, Post | ELISA | 8; first morning, days 23-31of cycle | 2.03 ± 0.32$ | Individual values | |
| 104 | Case/control | 58 | White, AA | N/A | Pre, Post | N/A | N/A | W: 2.2 ± 1.3 AA: 1.8 ± 1.1 | Mean, SD | |
| 64 | Descriptive multi-geographic Asian populations | 511 | Asian | 25-55 | Pre, Post | ELISA | 1; overnight, day of menstrual cycle recorded | Premeno: China 1.89; Japan: 1.67; Philippines:1.31 Postmeno: | GM | Menopausal status |

*Mutat Res*. Author manuscript; available in PMC 2013 September 03.

| Study reference | Main Topic of the study | N£ | Race | Age (years) | Menopausal Status | Assay | # Measures; methods of urine collection | 2/16 ratio | Statistics presented | Main analyses in the original paper |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | China 1.71; Japan 1.68; Philippines 1.8 | | |
| 65, 66* | Dietary factors; Brassica trial | 37 | White | >45 | Post | ELISA | 2; 24 hrs | 2.35 ± 1.33 | Mean, SD | Overall and by intervention |
| 4 | Family history of breast cancer | 64 | 91% white | Median: 50 | Pre, Post | ELISA | 1; spot | 2.13$ (Pre: 2.1 ± 0.8; Post: 2.2 ± 0.7) | Mean, SD | Menopause & age |
| 67 | Flaxseed | 15 | mostly white | 20-38 | Pre | ELISA | 3; 24 hrs, mid-luteal | 2.26 ± 0.19 | Mean, SE | Diet group |
| 68 | Case/control | 36 | Asian | Median: 54.8 | Pre, post | ELISA | 1; overnight | 2.0 ± 0.3 | Mean, SE | |
| 69 | Case/control | 64 | Multiracial | Median: 54.2 | Pre, post | ELISA | 1;spot | 1.72 ± 0.66 | Mean, SD | Menopausal |
| 70 | Case control | 326 | White, AA | N/A | Pre, post | ELISA | 1;spot | Pre: 1.9 ±1.0; post: 2.0 ± 1.0 | Mean, SD | |
| 99# | Osteopenia, Bone density | 59 | Korean | 55-60 | Post | GC-MS | 1; overnight | 0.17; 0.1# | Mean, SE | Bone density |
| 71 | Soy, isoflavones | 85 | Mostly Asian | Median: 33 | Pre | ELISA | Daily; 12 hrs | 2.0 ± 0.32 | Mean, SE | Diet |
| 72** | Bone density | 71 | N/A | 47-59 | Post | ELISA | 1; 24 hrs | 2.85 ± 1.73 | Mean, SD | Bone density |
| 73 | Soy intake | 16 | N/A | 18-40 | Pre | ELISA | 2; 48 hrs, mid luteal & mid follicular | 2.32 ± 1.11 | Mean, SD | Diet & OC use |
| 74 | Blood pressure | 54 | White/AA/Latino | 50-67 | Post | ELISA | 1; overnight | 1.5 | Mean | HRT status |
| 75 | Flax, genes | 132 | 97% white | 45-75 | Post | ELISA | 2; morning spot | 1.54 ± 0.75 | Mean, SD | Overall |
| 5, 76* | Physical activity | 146 | Chinese | 47.1 | Pre, Post | ELISA | 2; overnight | 1.2 ± 1.0 | Mean, SD | Race |
| 77 | Cancer incidence | 296 | N/A | >35 | Pre, Post | ELISA | 1; spot | Pre: 2.1; Post: 1.7 | Median | Menopausal |
| 78, 79# | OC & HRT | 67, 63 | N/A | Median: Pre= 26; Pos=55 | Pre, Post | ELISA | 2; 8 hrs overnight | Pre: 1.6 ± 0.12; 1.5 ± 0.1 Post: 2.1 ± 0.18; 1.67 ± 0.13 | Mean, 95% CI | Pre & Post HRT or OC |
| 80, 81# | Various hormonal treatment groups | 74 | N/A | Median: 54 | Post | ELISA | 2; 8hrs overnight | 1.15 ± 0.14; 1.5 ± 0.22; 1.2 ± 0.1; 2.0 ± 0.04 | Mean, 95%CI | HRT |
| 82, 83** | Genes/ bone density | 156 | White | Median: 63.5 | Post | ELISA | 1; 24 hrs | 2.1 ± 0.13 | Mean, SE | Genotype |

| Study reference | Main Topic of the study | N£ | Race | Age (years) | Menopausal Status | Assay | # Measures; methods of urine collection | 2/16 ratio | Statistics presented | Main analyses in the original paper |
|---|---|---|---|---|---|---|---|---|---|---|
| 84 | Soy intake in cancer survivors | 20 | N/A | Median: 56.2 | Post | ELISA | 4; 24 hrs | 1.92 (1.61-2.29) | GM, 95% CI | Overall, survivors, controls |
| 85 | Low fat diet, physical activity | 174 | Mostly White | 44-50 | Pre | ELISA | 2; spot | 2.3 ± 1.1 | Mean, SD | Treatment group |
| 86 | Breast density | 140 | White | 40-65 | Post | ELISA | 1; N/A | 0.98 (0.59-1.28) | Mean, range | Breast density |
| 100 | Sedentary and Physical Activity | 15 | N/A | Median: 29 | Pre | GS MS | 3; 24 hrs | 0.93 ± 0.62 | Mean, SD | Intervention |
| 87, 6 | Diet &lifestyle | 1881 | AA/White/Asian/ Hispanic | 45-54 | Pre, Peri | ELISA | 1; overnight, mostly follicular | AA: 1.65 ± 0.05 White:2.1 ± 0.04 China:1.9 ± 0.12 Hisp.:1.95±0.14 Japan:1.92± 0.09 | Mean, SE | Race |
| 88, 89 | Multiethnic | 33 | White/AA | 18-73 | Pre, Post | ELISA | 1; morning | White:2.25±0.89 AA: 1.42 ± 0.61 | Mean, SD | Race |
| 90, 91 | Multiethnic (pilot) | 125 23 | Chinese, AA, White | 45-75 55-64 | Post | ELISA | 1; morning | Chinese:1.63 (1.41-1.89); White+AA: 1.48 (1.27-1.84); 1.58 ± 0.2 | GM 95% CI Mean, SE | Race Breast cancer |
| 92 | Family history of breast cancer | 97 | White | 20-50 | Pre | ELISA | 1; morning, 5-9 days of cycle | 1.82 (1.49-2.15) | GM 95% CI | Family history |
| 93, 94 | Diet & exercise | 24 | N/A | Median: 31.5 | Pre | ELISA | 5; morning, mid follicular and mid luteal | 1.95 ± 0.18 | Mean, SE | Menstrual phase |
| 95 | Case/control | 426 | White | 50-64 | Pre, Post | ELISA | 1; spot | 1.6 (0.3-0.5); | Median ^ (5-95%) | HRT |
| 101, 102 | Menstrual cycle, soy isoflavones | 12 | N/A | Median: 26 | Pre | GC-MS | 3; 24 hrs | 18 ± 3.92 | Mean, SD | Diet, menstrual cycle |
| 103 | Soy diets | 18 | N/A | Median: 56.9 | Post | GC-MS | 2; 24 hrs | 1.33 ± 0.08 | Mean, SE | Overall & diet |

Abbreviations: GC-MS=Gas chromatography mass spectrometry, ELISA=Enzyme -linked immunosorbent assay, RIA=Radiometric ImmunoAssay, N/A=not indicated in publication, AA=African American, HRT=hormone replacement therapy, OC=oral contraceptive, NS=non-smokers, SMK=smokers; GM=geometric mean, SD=standard deviation, SE=standard error, CI=confidence intervals

£Number of subjects in control or non-treatment groups

^ratio reported only on a subset of the various populations included

* Partial overlap, 37 Whites in ref. 76 were published in ref. 66

** Partial overlap, 26 Whites were included in ref 83; (new data: 45)

+ mean was calculated from median; SD from ranges when full ranges were available

# 16/2 is reported in the original paper

& (3 on OC, 1 on HRT)

$ overall mean over 4 menstrual cycles was re-calculated