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Review

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 identified, 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 1.5–2.74, 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.

Contents

1. 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 activities; 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

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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 the 16α-OHE_1 in a mutually exclusive way [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 (HRT) 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 Ref. [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.

2. Materials and methods

2.1. Study identification and selection criteria

Original 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 1854 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, 32 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.

2.2. 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.

2.3. Laboratory methods

Earlier studies utilized the gas chromatography–mass spectrometry technique [45], a labor intensive method [46]. In 1994, Klug et al. [47] developed an Enzyme-Linked Immunosorbent Assay (ELISA) technique, 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 post-menopausal 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.

2.4. 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.

2.5. 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 post-menopausal 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 pre-menopausal women, on post-menopausal 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 h or more urine collection).

3. Results

3.1. 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 six studies used GC–MS [96–103], and one study [104] did not report the laboratory method. Self-defined ethnicity was reported in 30/43 data sets. Of these, 14 [6,53,59–63,65,66,82,83,86–89,93–97,104] 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