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Association of Estrogen Metabolism with Breast Cancer Risk in Different Cohorts of Postmenopausal Women

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Abstract

Endogenous estradiol and estrone are linked causally to increased risks of breast cancer. In this study, we evaluated multiple competing hypotheses for how metabolism of these parent estrogens may influence risk. Prediagnostic concentrations of estradiol, estrone, and 13 metabolites were measured in 1298 postmenopausal cases of breast cancer and 1524 matched controls in four separate patient cohorts. Median time between sample collection and diagnosis was 4.4–12.7 years across the cohorts. Estrogen analytes were measured in serum or urine by liquid chromatographic-tandem mass spectrometry. Total estrogen levels (summing all 15 estrogens/estrogen metabolites) were associated strongly and positively with breast cancer risk. Normalizing total estrogen levels, we also found that a relative increase in levels of 2-hydroxylation pathway metabolites, or in the ratio of 2-hydroxylation:16-hydroxylation pathway metabolites, were associated inversely with breast cancer risk. These associations varied by total estrogen levels, with the largest risk reductions occurring in women in the highest tertile. With appropriate validation, these findings suggest opportunities for breast cancer prevention by modifying individual estrogen metabolism profiles through either lifestyle alterations or chemopreventive strategies.

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Keywords

Estrogens / Metabolism; Breast Neoplasms; Postmenopause; Cohort Studies; Meta-Analysis

Introduction

Extensive epidemiological, laboratory, and clinical evidence have demonstrated that elevated endogenous estrogen levels are associated with increased postmenopausal breast cancer risk (1–5). Laboratory studies have assessed the roles of parent estrogens (estradiol and estrone) and their metabolites in carcinogenesis (2,4,5). Estrone and estradiol are irreversibly hydroxylated at the C-2, C-4, or C-16 positions of the steroid ring, leading to a cascade of metabolites (Figure 1). As reviewed by Yager and Davidson (4), two major hypotheses about the carcinogenicity of estrogen metabolites have emerged from decades of experimental research: 1) 16-hydroxylation metabolites, and particularly 16 α -hydroxyestrone, can bind covalently to the estrogen receptor (ER) with moderate to strong mitogenic effects; and 2) 2- and 4-hydroxylation catechol estrogen metabolites can be oxidized into mutagenic quinones that form DNA adducts and lead to oxidative DNA damage (1,2,4,6–8). While there have been spirited debates as to whether mitogenic or mutagenic mechanisms are more important in estrogen-mediated carcinogenesis, both are thought to be likely to contribute (2,4).

Prospective epidemiologic studies over the last two decades have consistently linked elevated circulating estradiol and estrone to increased postmenopausal breast cancer risk (3). However, until very recently none has comprehensively addressed the specific hypotheses noted above or overall relationships of estrogen metabolism to breast cancer risk (9). This has been primarily because there were no accurate, reliable biochemical methods to measure the full range of these metabolites in human populations, particularly at low concentrations characteristic of postmenopausal women (9).

Recently, highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been developed to simultaneously measure parent estrogens and 13 of their metabolites in serum (10) and urine (11). We used this assay in four case-control studies of postmenopausal breast cancer conducted within prospective cohorts (12–15). Although risk estimates for individual estrogen metabolites varied, each study suggested that enhanced 2-hydroxylation of parent estrogens was associated with reduced breast cancer risk. Given the relatively small sample sizes in each study (215–407 cases), analysis and interpretation were limited. Thus, we pooled individual data from these studies in an analysis including 1298 cases and 1524 controls to more robustly test the role of estrogen metabolism in breast carcinogenesis.

Materials and Methods

Data Collection

Four published studies (12–15) of breast cancer risk in postmenopausal women used the same comprehensive assay of endogenous estrogens and their metabolites, allowing individual participant data to be pooled in this analysis. The studies were based on cohorts

with prospectively collected serum or urine from women not currently taking exogenous hormones:

1. The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), a multicenter US cancer screening study, randomized 39,116 women to the screening arm (1993–2001). Within this cohort a nested case-control study was conducted of 277 cases and 423 controls, matched on age and period of baseline blood collection (12).
2. The Columbia Missouri Serum Bank recruited 6,915 women living in or around Columbia, MO (1977–1987) as part of the NCI's Biological Markers Project to identify serum markers of breast cancer. Within this cohort a nested case-control study was conducted of 215 cases and 215 controls, matched on age, season and time of day of blood collection, and type of and years since menopause (13).
3. The Breast and Bone Follow-up to the Fracture Intervention Trial (B~FIT), is comprised of 15,595 postmenopausal women screened for FIT, a randomized study of alendronate, at 10 US clinical centers where baseline blood samples were collected (1992–1993). The long-term follow-up for breast cancer identified 407 breast cancer cases and 487 controls, randomly selected within 10-year age and clinical center strata (14).
4. The Shanghai Women's Health Study (SWHS), a population-based cohort, enrolled 74,942 women from seven communities in Shanghai, China and collected spot urines (1997–2000). A nested case-control study was conducted of 399 cases and 399 controls, matched on age, date and time of urine collection, time since last meal and antibiotic use (15).

All cases comprised invasive breast cancers, with the exception of Columbia, MO and B~FIT which included 15 and 54 *in-situ* cancers, respectively. Details of study design, informed consent, ethics approvals, and covariate definitions are in the original study publications (12–15). Additional ethics approval was not required for this analysis.

Laboratory assay

Stable isotope dilution LC-MS/MS was used to measure total (conjugated, including both glucuronidated and sulfated, plus unconjugated) concentrations of 15 estrogens (two parent estrogens and 13 estrogen metabolites) at a single laboratory (Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, MD), as previously described (10,11,16,17). While PLCO, Columbia, and B~FIT measured serum, SWHS measured urine and normalized to creatinine levels. In all four studies, laboratory coefficients of variation from masked quality control samples were <5% for all individual analytes (12–15).

Statistical Methods

For each study, we used logistic regression to model linear associations between the log-concentration of each analyte and postmenopausal breast cancer risk. We report odds ratios (ORs) and 95% confidence intervals (CIs) comparing 90th vs. 10th percentiles. ORs were obtained by fitting a model with log-analyte level as an independent continuous variable and

Results

The characteristics of breast cancer cases (N=1298) and controls (N=1524) in this analysis are presented in Table 1. Within each study, cases and controls had similar characteristics, but cases tended to have slightly older ages at first birth, younger ages at menarche, and higher BMIs than controls. Median time between blood/urine collection and breast cancer diagnosis was 4.4, 5.6, 6.2, and 12.7 years for PLCO, SWHS, B~FIT, and Columbia, respectively.

Median (10th–90th percentile) concentrations of two parent estrogens and 13 metabolites are provided in Table 2. Parent estrogens, Path-2, Path-4, and Path-16 metabolites comprised 35%, 20%, 3%, and 42% of total estrogen (sum of all 15 concentrations), respectively (Figure 2).

Higher total estrogen levels were strongly associated with increased postmenopausal breast cancer risk ($P<0.001$). The OR of breast cancer, when comparing women in 90th vs. 10th percentiles of total estrogen, was 1.48 (95%CI:1.20–1.82; Figure 3). Excluding BMI from the model produced similar results (OR=1.52, 95%CI:1.25–1.86). Most of the 15 individual analytes were also significantly associated with risk, consistent with the observation that most analytes were highly correlated with each other (median Pearson correlation coefficient=0.67; interquartile range:0.57–0.76; Supplementary Tables 1A–B). Comparing the highest to the lowest quintile of total estrogen, the OR for women in the highest quintile was 1.69 (95%CI:1.30–2.21; Supplementary Table 2A), consistent with and somewhat higher than that estimated in Figure 3 under the assumption of linearity. The ORs for women in the 2nd, 3rd, and 4th quintiles were 1.17 (0.9–1.53), 1.36 (1.05–1.78), and 1.31 (1–1.72), respectively (Supplementary Table 2A).

We examined the effect of changing the proportion of each metabolite or pathway, relative to total estrogen level, while holding total estrogen concentration constant (Supplementary Table 2B). In a model that adjusted for total estrogen level, we found that an elevated Path-2:Total ratio was associated with a statistically significant reduced breast cancer risk (OR for 90th vs. 10th percentile=0.61, 95%CI:0.46–0.80, $P<0.001$; Figure 4). Elevated proportions of Path-4 and Path-16 metabolites were associated with non-statistically significant reductions and increases in risk, respectively. These patterns were seen in each study individually (Supplementary Table 3), in analyses with single studies removed (Supplementary Table 4), and in random effects meta-analysis (Supplementary Table 5).

We also considered the Path-2:Path-16 ratio, a common metric in prior studies (9), and found that individuals with higher ratios had a significant reduction in risk both before (OR for 90th vs. 10th percentile=0.65, 95%CI:0.54–0.79, $P<0.001$; Figure 3) and after (OR=0.66, 95%CI:0.52–0.84, $P<0.001$; Figure 4) adjusting for total estrogen. This pattern was seen in each study individually (Supplementary Table 3), in analyses with single studies removed (Supplementary Table 4), and in random effects meta-analysis (Supplementary Table 5). We also found an inverse correlation between the Path-2:Path-16 ratio and total estrogen in each study (Supplementary Figure 1). Risk estimates were similar after adjusting for only parent estrogens, in which the OR comparing 90th vs. 10th percentiles of the Path-2:Path-16 ratio

was 0.69 (95% CI:0.55–0.87); adjusting for both parent and total estrogen levels, the OR was 0.67 (95% CI:0.53–0.85). The ratio of 4-hydroxylation pathway catechols to 4-hydroxylation pathway methylated catechols was not strongly associated with risk (OR=1.15, 95% CI:0.98–1.36) (Supplementary Table 3).

We tested if the inverse relation of either the Path-2:Path-16 or Path-2:Total ratio with breast cancer risk varied by total estrogen concentration or breast cancer risk factors. We present the results only for the Path-2:Path-16 ratio here, as results for the Path-2:Total ratio were similar. Total estrogen was the only factor to have a statistically significant effect (P-interaction=0.003), with the strongest inverse association between the Path-2:Path-16 ratio and breast cancer risk in women with the highest total estrogen levels (Supplementary Table 6). Among women in the bottom, middle, and highest tertiles of total estrogen, the respective ORs (95% CI) were 1.08 (0.68–1.71), 0.70 (0.44–1.12), and 0.43 (0.27–0.69) (Supplementary Table 6). This pattern was also apparent in the nine groups of women defined by tertiles of total estrogen and tertiles of the Path-2:Path-16 ratio (Supplementary Table 7). BMI was the only risk factor identified as potentially being associated with Path-2:Path-16 ratio (p=0.04), with the inverse association strongest in the SWHS population (p=0.003) (Supplementary Table 8).

Discussion

We evaluated the role of endogenous estrogen metabolism in postmenopausal breast cancer risk using data collected from four studies with prospectively collected serum or urine samples. All studies found that increased parent and total estrogen concentrations were strongly associated with increased breast cancer risk. Our analysis further demonstrated that for a given level of total estrogen, increased levels of 2-hydroxylation pathway metabolites were associated with reduced risk. While the association between elevated parent estrogens and increased postmenopausal breast cancer risk has been well established, our robust findings of reduced risk associated with estrogen metabolism into the 2- as opposed to the 16-hydroxylation pathway also support the long-standing hypothesis that much of the carcinogenicity of estrogens may relate to their mitogenic effects in enhancing cell proliferation, either leading to increased opportunity for random mutations or acting as late-stage promoters. While previous studies have attempted to address mitogenic vs. mutagenic hypotheses of estrogen-mediated breast carcinogenesis, they only measured two metabolites (2-hydroxyestrone and 16 α -hydroxyestrone via direct enzyme immunoassays), were unable to comprehensively measure pathways, and produced conflicting and generally non-significant results (9).

The lack of any evidence of a particular adverse effect of catechol estrogens in our analysis is inconsistent with the other long-standing hypothesis that the 2- and 4-hydroxylation pathways produce genotoxic metabolites that can form DNA adducts, thereby acting as direct carcinogens (8). Two (12,13) of the four (12–15) cohort studies in this analysis showed some evidence of increasing risk with an increasing ratio of 4-hydroxylation pathway catechols:4-hydroxylation pathway methylated catechols (Supplementary Table 3), which would be consistent with this hypothesis (i.e., removal of genotoxicity by conversion

of one hydroxy group to a methoxy group). However, the other two studies (14,15) and this combined analysis did not confirm this association; this issue requires further attention.

The lower breast cancer risk associated with higher 2-hydroxylation pathway metabolite levels, conditioned on total estrogen, is promising on several fronts. First, it is a strong association, with women in the highest decile of the 2-hydroxylation:16-hydroxylation ratios having ~30–40% lower risk than those in the lowest decile. In addition, the beneficial effect appears to increase with increasing total estrogen levels. If confirmed, the ~50% risk reduction associated with extensive 2-pathway hydroxylation among women with the highest total estrogen levels might offer an opportunity for targeted intervention. Levels of these ratios may be modifiable by lifestyle changes as they have been inversely associated with BMI (19) and positively associated with physical activity (20,21). Moreover, the higher ratios in Asian populations, with lower breast cancer risk (15), decline when these populations migrate West (22). Our findings require replication and further investigation to assess potential causality in these relationships, but these estrogen metabolite ratios could be modifiable risk factors and targets for prevention.

Major strengths of this study are the prospectively collected biologic samples from four high-quality cohort studies, and the opportunity to assay metabolites in the same laboratory. Furthermore, we measured total concentrations of estrogens, not just unconjugated concentrations, using newly developed, reliable, sensitive and specific LC-MS/MS assay for estrogens and estrogen metabolites. The sample preparation method for the LC-MS/MS assay employed in our studies removed sulfate and glucuronide moieties from conjugated forms of the estrogens and estrogen metabolites and released unconjugated estrogens, such as estradiol, from sex hormone binding globulin (SHBG). As we did not measure serum SHBG in these cohorts, we were unable to estimate non-SHBG bound estradiol as has been reported in prior studies using conventional immunoassays (3). One limitation is the variability in absolute values of analytes across studies. However, this is not unusual for metabolite analyses and has less of an impact on ratios. Another limitation is that one (15) of the four (12–15) studies measured urinary, rather than serum, estrogens. However, studies of parent estrogens have consistently found similar relative risks for breast cancer in blood or urine (23). While most risk factors and other metrics were similar among the four studies, there were exceptionally strong correlations between all of the analytes and a comparatively narrow range of total estrogen levels within the PLCO study. These differences may be attributable to variability in LC-MS/MS calibration and standardization, but may be due to other factors as well. In sensitivity analyses excluding each of the studies one at a time, findings were consistent with those observed for all studies combined, suggesting robust results. The four studies had different procedures for selecting controls. While Columbia, MO and SWHS matched each case with a control alive at the time of diagnosis, PLCO selected controls among women alive at the end of the study and B-FIT selected controls among all women, with the latter two selection procedure selections leading to potential survivor bias. Finally, hormone receptor status was obtained only on a fraction of cases. Among postmenopausal women in prior reports, the strongest effects for endogenous estradiol have been observed for ER-positive tumors (24–26). About 81% of breast cancers among US women aged 50+ years are ER-positive (27). Additional studies will be needed to address heterogeneity of estrogen metabolite associations by ER status.

We measured estrogens in a single specimen prior to clinical disease, consistent with the methods for the majority of postmenopausal breast cancer studies (3). A single measure of estradiol was recently found to predict postmenopausal breast cancer risk for up to 16–20 years (24). The timing of sampling in our studies, most ranging from 4–13 years prior to diagnosis, seems ideal to characterize the possible role of metabolites as tumor promoting mitogens. If initiation of breast cancer occurs decades before diagnosis, then our design may not provide a strong test of metabolic profiles associated with initiation. Future laboratory research along with work in large epidemiologic cohorts would be needed to clarify the genetic determinants of estrogen metabolism as well as the mechanisms by which estrogen metabolites may contribute to each phase of the carcinogenic process.

In summary, we demonstrated that among postmenopausal women, relative increases in levels of 2-hydroxylation pathway metabolites are significantly associated with reduced breast cancer risk after adjusting for total estrogen levels. Whether this relationship is causal remains to be determined, but plausible biologic explanations are supported by laboratory, epidemiological and clinical evidence (1–5). If judged to be causal, these findings could support the notion of altering estrogen metabolism through lifestyle modifications or chemopreventive strategies as a means of breast cancer prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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RGZ and XX hold patents and receive royalties pertaining to the estrogen metabolite assay.

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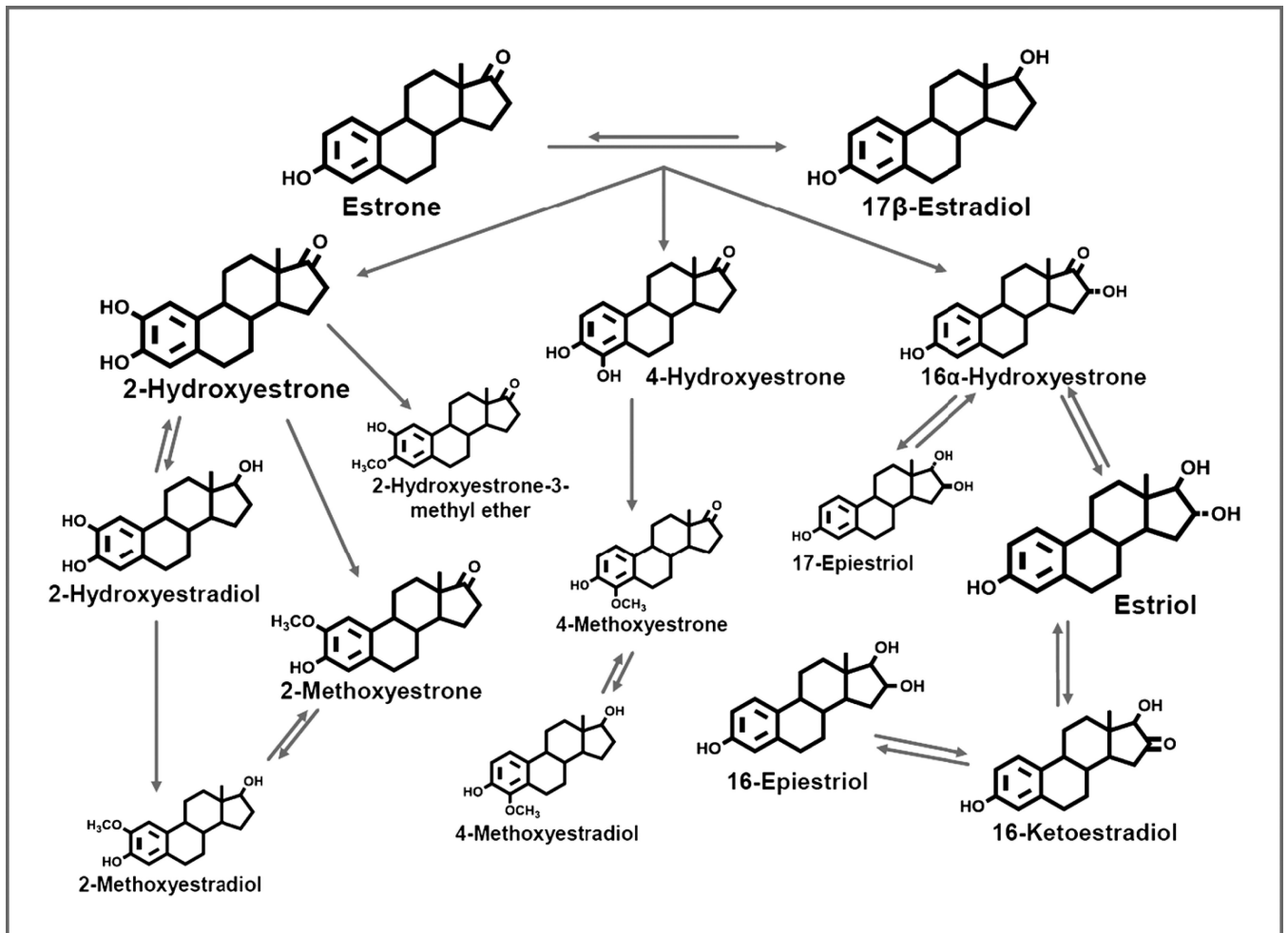


Figure 1. Schematic of estrogen metabolic pathway. Sizes of chemical structures as depicted are based on the relative abundance of the estrogen/estrogen metabolite.

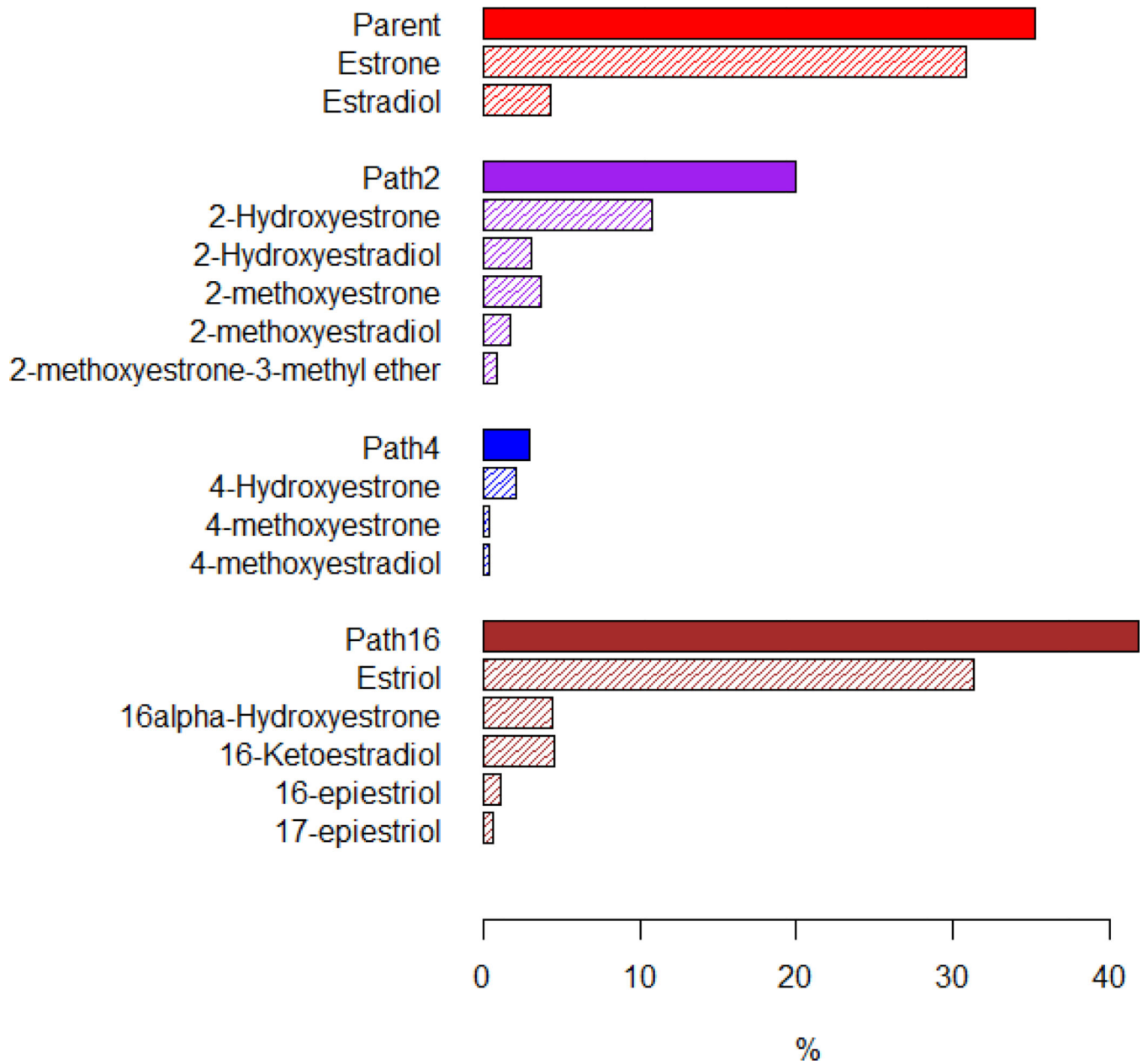


Figure 2. Percentage of total estrogen (sum of 15 estrogens/estrogen metabolites) in each of the metabolic pathway groups and in each of the individual estrogens/estrogen metabolites. The proportions for the parent estrogens, 2-hydroxylation pathway (Path2), 4-hydroxylation pathway (Path4), and 16-hydroxylation pathway (Path16) estrogen metabolites sum to 100%.

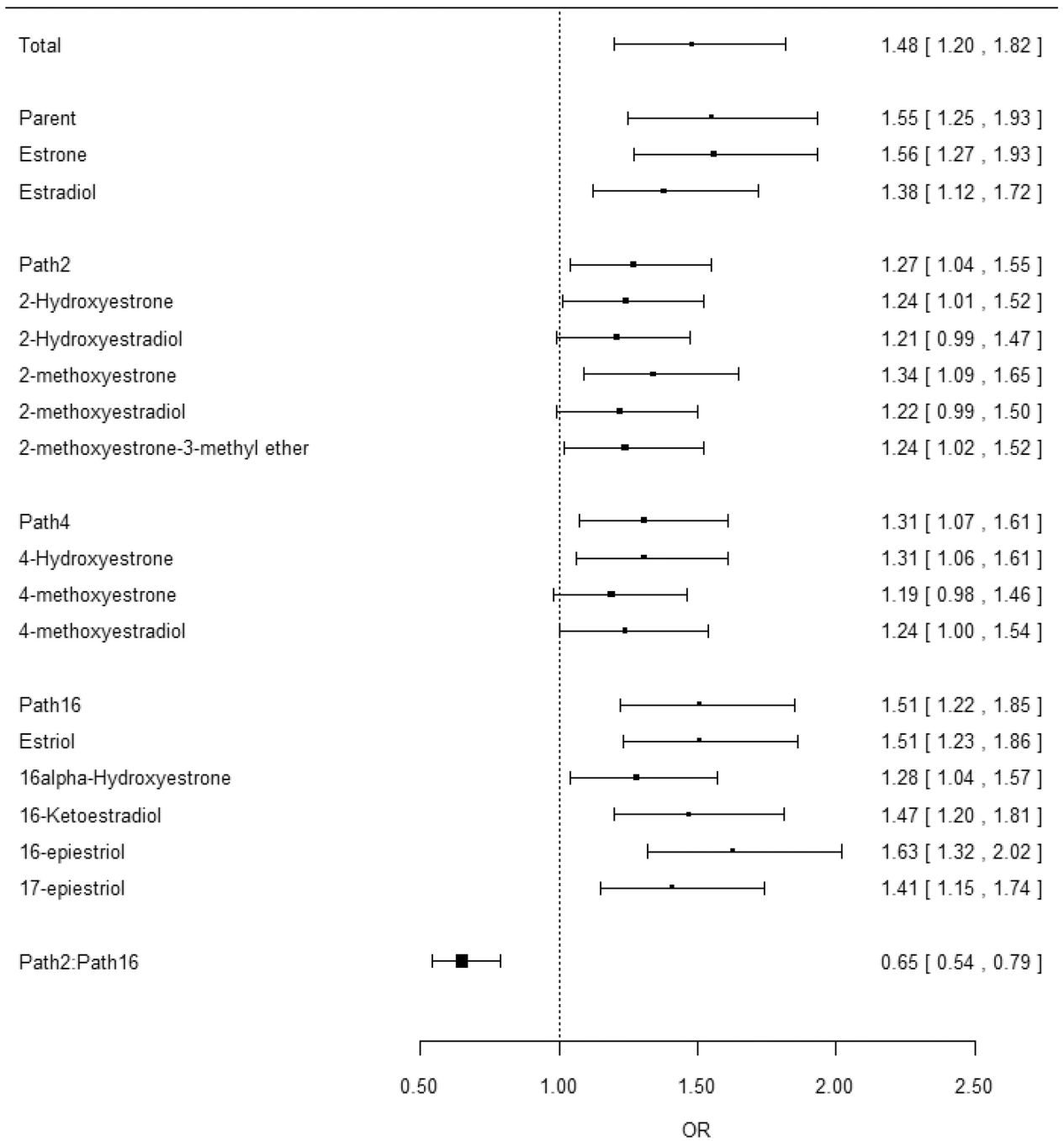


Figure 3. Odds ratios and 95% confidence intervals comparing the risk of breast cancer in individuals with a higher analyte or pathway concentration (90th percentile) to individuals with a lower concentration (10th percentile). Odds ratios were modeled by logistic regression with log-concentration as the independent variable and without adjusting for total estrogen level.

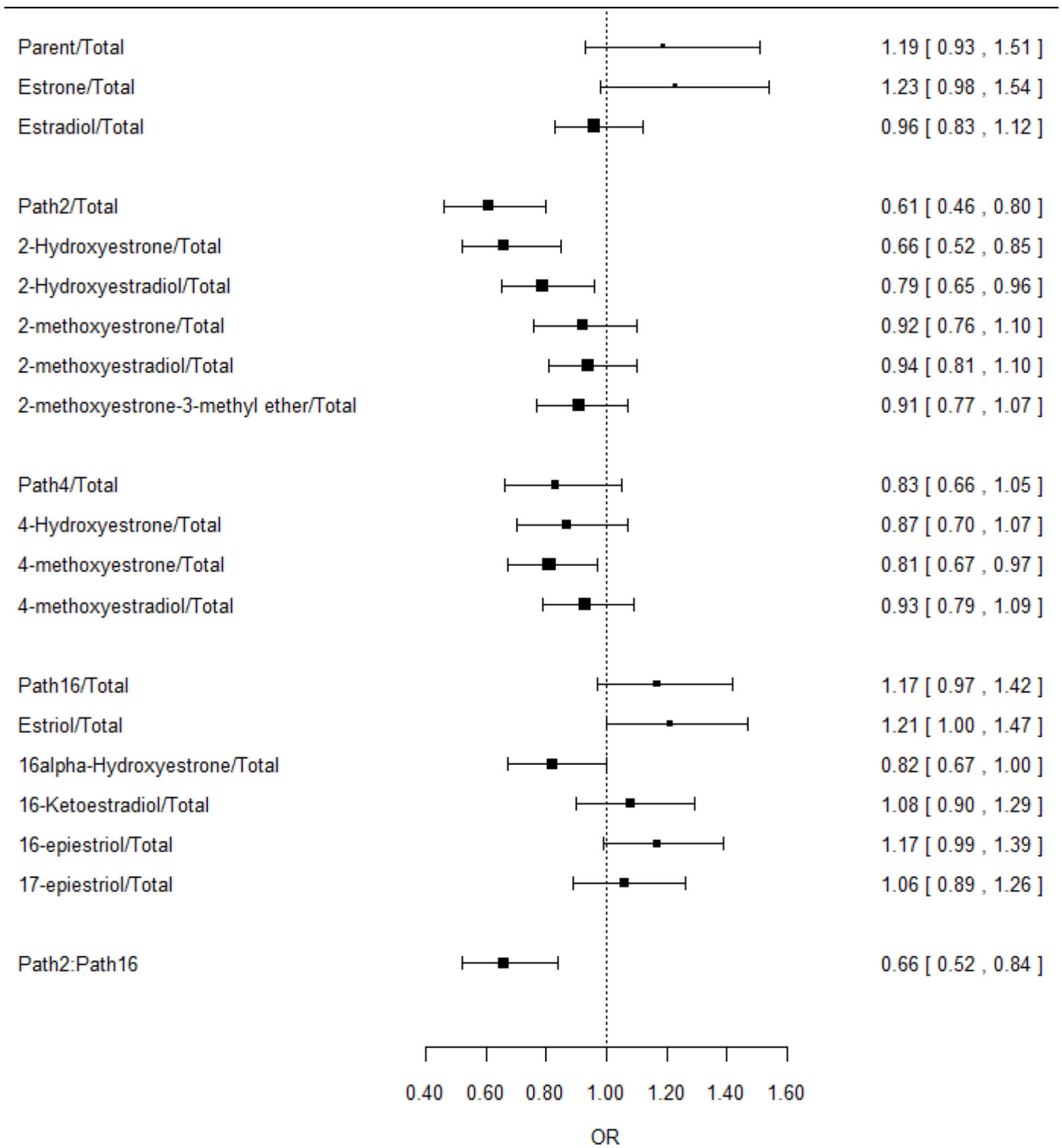


Figure 4. Odds ratios and 95% confidence intervals comparing the risk of breast cancer in individuals with a higher analyte:total or pathway:total ratio (90th percentile) to individuals with a lower ratio (10th percentile). Odds ratios were modeled by logistic regression with ratio as the independent variable and adjusting for total estrogen level.

Table 1

Baseline characteristics.

Characteristic	PLCO		Columbia, MO		B-FIT		SWHS	
	Controls N = 423	Cases N = 277	Controls N = 215	Cases N = 215	Controls N = 487	Cases N = 407	Controls N = 399	Cases N = 399
Age (years)								
<55	0% [‡]	0%	29%	28%	0%	0%	22%	2%
55–<65	55%	55%	43%	48%	34%	29%	52%	53%
65	45%	45%	28%	24%	66%	71%	26%	25%
BMI kg/m²								
<25	38%	31%	43%	40%	39%	34%	56%	50%
25–<30	35%	41%	38%	41%	34%	35%	35%	41%
30	26%	26%	19%	18%	27%	30%	9%	10%
Ever Pregnant[‡]								
No	9%	9%	16%	17%	9%	13%	4%	5%
Yes	91%	91%	84%	83%	91%	87%	96%	95%
Age at First Birth								
<20	16%	13%	16%	11%	10%	9%	14%	9%
20–24	47%	44%	31%	40%	43%	39%	37%	35%
25–29	19%	22%	27%	23%	25%	30%	34%	37%
30	9%	12%	10%	9%	13%	10%	10%	14%
Never pregnant	9%	9%	16%	17%	9%	13%	4%	5%
Age at Menopause								
< 45	10%	10%	24%	25%	26%	23%	16%	13%
45–50	23%	23%	29%	25%	26%	28%	43%	38%
50	50%	48%	47%	51%	47%	48%	40%	46%
Age at Menarche								
13	71%	76%	72%	73%	71%	74%	18%	24%
>13	29%	24%	28%	27%	25%	24%	82%	76%
Ever HT Use								
No	61%	67%	95%	95%	67%	63%	96%	94%

Characteristic	PLCO		Columbia, MO		B-FIT		SWHS	
	Controls N = 423	Cases N = 277	Controls N = 215	Cases N = 215	Controls N = 487	Cases N = 407	Controls N = 399	Cases N = 399
Yes	39%	33%	5%	5%	33%	37%	4%	6%
Ever smoke								
No	60%	53%	74%	73%	52%	53%	95%	97%
Yes	40%	47%	26%	27%	47%	46%	5%	3%
Race								
White	88%	88%	100%	100%	95%	95%	0%	0%
Other	12%	12%	0%	0%	5%	5%	100%	100%

[†] PLCO and B-FIT report number of live births, while Columbia, MO and SWHS report number of pregnancies.

[‡] Percentages are reported as the percentage of the total population, where the total includes those individuals missing values. The table does not include “Missing” categories, and therefore percentages need not sum to 100%.

Abbreviations: BMI, body mass index; HT, hormone therapy.

Median (10th – 90th percentile) metabolite concentrations are reported for each study. Colombia, B~FIT, and PLCO report serum concentrations in pmol/L, while SWHS reports urine concentrations in pMol/mg creatinine

Table 2

Estrogen/Estrogen Metabolite	PLCO		Colombia, MO		B~FIT		SWHS	
	Median	(Inter-decile range)	Median	(Inter-decile range)	Median	(Inter-decile range)	Median	(Inter-decile range)
Estrone	393.5	(365.1–444.1)	242.5	(101.6–702.1)	306.4	(118.7–828.8)	5.8	(2.4–14.2)
Estradiol	36.2	(32.7–38)	25	(10.9–77.2)	41.2	(19–95)	1.2	(0.5–3)
2-Hydroxyestrone	69	(64.3–74.9)	74.4	(64.4–146.5)	64.3	(38.7–142)	4.9	(2.3–11)
2-Hydroxyestradiol	35.9	(33.6–39)	23.6	(12.3–47.9)	12.5	(7.5–25.8)	1.2	(0.6–2.7)
2-Methoxyestrone	12.5	(11.6–13.4)	42.5	(22.4–92.1)	29.2	(14–62.1)	1.1	(0.4–2.7)
2-Methoxyestradiol	7.7	(7.1–8.2)	14.3	(6.7–34.3)	13.5	(7–26.9)	0.5	(0.2–1.5)
2-Methoxyestrone-3-methyl ether	3.4	(3.2–3.7)	6	(2–16.5)	4.1	(2.4–12.4)	0.3	(0.1–0.7)
4-Hydroxyestrone	22.1	(20.7–24.1)	18.5	(13.8–34)	10.7	(6.5–21.6)	0.7	(0.3–1.6)
4-Methoxyestrone	3.5	(3.2–3.8)	3.5	(1.2–7.3)	3.9	(2.2–10.1)	0.1	(0.1–0.4)
4-Methoxyestradiol	2.8	(2.6–3.1)	1.9	(1–5.4)	3	(1.3–6.2)	0.1	(0–0.2)
Estril	440	(404.6–487.9)	278.2	(166.5–597.2)	255.1	(133.1–620.2)	5.5	(2.6–12.4)
16 α -Hydroxyestrone	39.2	(36.1–42.6)	37.5	(27.6–78.9)	28.6	(17.8–70.3)	1.5	(0.7–3.2)
16-Ketoestradiol	36.5	(34.4–39.9)	31.9	(20.1–67.8)	32.8	(18.3–104.2)	1.6	(0.7–3.8)
16-Epiestriol	3.5	(3.2–3.8)	5.7	(2.1–19.7)	4.2	(2–16.1)	0.6	(0.2–1.5)
17-Epiestriol	1.3	(1.3–1.5)	2.3	(1.1–7.6)	3.5	(1.4–11)	0.4	(0.1–0.9)