The relationship between physical activity and 2-hydroxyestrone, 16α-hydroxyestrone, and the 2/16 ratio in premenopausal women (United States)

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Abstract

Objectives: Estrogen is metabolized in the body through two mutually exclusive pathways yielding metabolites with different biological activities: the low estrogenic 2-hydroxyestrone (2-OHE1) and the highly estrogenic 16α-hydroxyestrone (16α-OHE1). The ratio of these metabolites (2/16) may be predictive of risk for developing breast cancer. Early evidence has demonstrated that exercise may alter estrogen metabolism to favor the weak estrogen, 2-OHE1.

Methods: Seventy-seven eumenorrheic females completed physical activity logs for two weeks prior to providing a luteal phase urine sample. Concentrations of 2-OHE1 and 16α-OHE1 were measured and the 2/16 ratio computed. Hierarchical regression, controlling for age and body mass index (BMI), was used to determine relationships between estrogen metabolites and daily physical activity.

Results: Regression analyses indicated significant positive relationships between physical activity and 2-OHE1 and the 2/16 ratio (p < 0.05) that appears to be independent of BMI. 16α-OHE1 was not significantly related to physical activity.

Conclusion: These results indicate that physical activity may modulate estrogen metabolism to favor the weak estrogen, 2-OHE1, thus producing a higher 2/16 ratio. This alteration in estrogen metabolism may represent one of the mechanisms by which increased physical activity reduces breast cancer risk.

Introduction

The International Agency for Research on Cancer (IARC) [1] has identified body fat and physical inactivity as the “most important avoidable causes” of breast cancer. The evidence that physical inactivity is directly related to breast cancer risk continues to accumulate [2–8]. Epidemiological studies [9–11] have shown that the risk for breast cancer is hormonally mediated and may be caused by years of estrogen exposure. Physical activity has been linked to decreased risk for breast cancer possibly due to the ability of physical activity to alter estrogen exposure [12, 13].

The predominant estrogen produced by the ovaries is 17β-estradiol. Enzymes in mammary cells convert estradiol to estrone, which can subsequently be hydroxylated at positions 2 and 16α [14–16]. 2-Hydroxyestrone (2-OHE1) is considered a weak estrogen due to its rapid methylation, rapid clearance rate, weak binding affinity for the estrogen receptor, and antiproliferative effect on mammary cells [17–21]. 16α-Hydroxyestrone (16α-OHE1) demonstrates estrogenic properties through covalent bonding with the estrogen receptor and stimulation of cell proliferation [22–25]. Due to the opposing actions of these metabolites, the ratio of 2-OHE1 to 16α-OHE1 (2/16 ratio) has been identified as a possible determinant of risk for breast cancer [26]. A low 2/16 ratio has been associated with increased risk for breast cancer in some [26–31] but not all investigations [32–35]. Evidence exists that estrogen metabolites can be modified by human behaviors such as smoking.
[36–38], hormonal contraceptives [39, 40], body fat [41– 43], and diet [44–46]. Physical activity is a modifiable human behavior that may alter estrogen metabolism but research is limited. Higher resting levels of 2-hydroxyestrogens have been found in female athletes as compared with nonathletic controls [41, 42, 47–49] while the level of 16α-OHE1 remained unaltered [47]. If physical activity does contribute to elevated levels of 2-hydroxylation, this altered estrogen metabolism may provide a mechanism for decreased breast cancer risk associated with physical activity. Early research examining the link between exercise and estrogen metabolites however has been limited by design flaws including small sample size [41], presence of menstrual dysfunction, and low adiposity [47]. We hypothesized that higher levels of physical activity would be correlated with a higher concentration of 2-OHE1 and a higher 2/16 ratio. If a biochemical relationship between physical activity and breast cancer risk reduction is found, the impact on motivating women to adhere to a regular program of exercise may be profound. Further, the 2/16 ratio may represent a biological marker of risk reduction that can be incorporated into prescribing exercise programs of specific intensity and duration.

Materials and methods

Recruitment and eligibility

Female participants were recruited from a database of 3300 individuals from a previous breast cancer investigation (unpublished). Women from northern Colorado who met the inclusion criteria were contacted by mail (n = 224). Eighty-six females volunteered to participate in this study. Inclusion criteria were based on the following: eumenorrheic (menstrual cycles consistently every 26–32 days for a minimum of one year), non-smoker for a minimum of six months, no hormonal contraceptives for at least three months plus a return to normal menstrual cycles (eumenorrheic for three months), body mass index (BMI) between 18 and 30, non-vegetarian, not pregnant or lactating, and no metabolic disorders (thyroid, kidney, liver, diabetes). Additionally, African American women were eliminated because of the prevalence for polymorphisms in the gene producing the 2-hydroxylation enzyme [50].

Data collection

All volunteers who met the eligibility criteria provided a urine sample during the luteal phase of the menstrual cycle (mean = 5 ± 1.6 days prior to onset of menses). To ensure that the sample was obtained during the luteal phase of the cycle, all participants were asked to report the number of days between providing the urine sample and the onset of menses. Diet records were recorded on two weekdays and one weekend day for the two weeks prior to collecting the urine sample. A physical activity log was developed based on previously published criteria [51]. Logs were maintained daily for two weeks prior to the scheduled urine sample collection. The activity logs included household, work, leisure, exercise, and sport related physical activity.

Urine collection and laboratory analysis

Participants collected a first morning urine sample in a specimen cup containing ascorbic acid (1 mg/ml) to prevent oxidation of the labile metabolites. The urine samples were immediately refrigerated, transported on ice, then labeled and stored at −80°C until analysis. Estrogen metabolites were measured using a competitive, solid-phase enzyme immunoassay (EIA) (ESTRAME™ 2/16, ImmunaCare Corp, Bethlehem, PA, USA) [52]. The frozen urine samples were brought to room temperature before testing. Since the samples were from premenopausal women, all samples were diluted 1:4 prior to testing with manufacturer-supplied diluent. 2-OHE1 is found in the urine as 3-glucuronide and 16α-OHE1 is in the form of 3,16α-glucuronide. Prior to recognition by monoclonal antibodies, glucuronic acid and sulphate are removed by incubation with the deconjugating enzymes, β-glucuronidase and arylsulphatase, respectively. Kinetic readings (405 nm) were taken every 2 min for 20 min using a Thermomax Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The sample results were determined from a calibration curve derived from the six standards supplied with the kit (0.625–15 ng/ml). Manufacturer-supplied as well as in-house controls were used with all assays. The samples, controls, and standards were assayed in triplicate per manufacturer’s recommendation. Any samples outside of the range of the standard curve or with a coefficient of variation (cv) greater than 10% were re-assayed. Fourteen samples were reanalyzed for 2-OHE1 and nine samples for 16α-OHE1. Overall intra-assay cv’s for 2-OHE1 and 16α-OHE1 were 4.5 and 4.3, respectively and inter-assay cv’s were 4.7 and 7.0, respectively. All ESTRAME™ 2/16 kits were from the same lot and were used within two weeks of delivery. Urinary 2-OHE1 and 16α-OHE1 levels were normalized to urinary creatinine concentration and expressed in ng/mg creatinine. Creatinine was measured in duplicate by colorimetric assay (Sigma, St. Louis, MO, USA). The