The Effects of Physical Exercise on Selected Markers of Estrogen Metabolism Linked to Breast Cancer Risk in Premenopausal Women

Laura Behrends

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Abstract
Background: Physical activity is linked to breast cancer risk reduction in women, yet the mechanism remains largely unknown. Possible causes for this association have been hypothesized to include change in endogenous estrogen production, estrogen metabolism, circulating concentrations of peptide hormones and growth factors, obesity, central adiposity, and immune function. Recent investigations in estrogen metabolism in women have brought to light a new possibility for the association between physical activity and breast cancer risk to be related to 2-hydroxyestrone metabolism increases with exercise, relative to 16α-hydroxyestrone. This metabolite pathway has been studied as a mechanism for postmenopausal breast cancer risk, but there are few studies concerning eumenorrheic, premenopausal women. The purpose of this systematic review is to evaluate the most current research on this topic.

Methods: An exhaustive search of available medical literature concerning estrogen metabolism, breast cancer risk, physical activity, and premenopausal females was conducted. The reviewed studies were limited to randomized controlled trials, and prospective and retrospective cohort investigations.

Results: The six articles included in this review showed either non-significant changes in estrogen metabolism to favor the anti-estrogenic 2-hydroxyestrone pathway, or no change at all in estrogen metabolism as a result of physical activity. The only investigation reaching statistically significant results was a retrospective cohort study relying on self-reports of physical activity. The six studies reviewed demonstrate vastly different inclusion and exclusion criteria, as well as intervention protocols, causing comparison between studies to be imprecise.

Conclusion: Current research has yet to identify the cause of the association between physical activity and reduced breast cancer risk as related to changes in estrogen metabolism. Further investigation with large randomized controlled trials with objectively measuring physical activity, utilizing standardized methods of gathering and analyzing data is needed.

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Physical Activity, Breast Cancer, Estrogen Metabolism, Premenopausal Women

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The Effects of Physical Exercise on Selected Markers of Estrogen Metabolism
Linked to Breast Cancer Risk in Premenopausal Women

Laura Behrends

A Clinical Graduate Project Submitted to the Faculty of the
School of Physician Assistant Studies
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Clinical Graduate Project Coordinators: Annjanette Sommers MS, PAC & Rob Rosenow PharmD, OD
Biography

Laura Behrends is a native of Woodland, California where she graduated from the University of California Santa Barbara with Highest Honors with a major in Psychology and a minor in Spanish. After completion of her undergraduate degree, she spent a year in the surgery department of Santa Barbara Cottage Hospital, and then worked as a medical assistant at Sansum Clinic in Santa Barbara for 4 years prior to her admission to Pacific University PA Program. During the program, Laura enjoyed spending some time in Costa Rica improving her medical Spanish and traveling all over the country. After graduation Laura plans to pursue a career in Family Medicine, and continue to create her unique cupcake delights so that she can open her own Cupcakery when she retires.
Abstract

**Background:** Physical activity is linked to breast cancer risk reduction in women, yet the mechanism remains largely unknown. Possible causes for this association have been hypothesized to include change in endogenous estrogen production, estrogen metabolism, circulating concentrations of peptide hormones and growth factors, obesity, central adiposity, and immune function. Recent investigations in estrogen metabolism in women have brought to light a new possibility for the association between physical activity and breast cancer risk to be related to 2-hydroxyestrone metabolism increases with exercise, relative to 16α-hydroxyestrone. This metabolite pathway has been studied as a mechanism for postmenopausal breast cancer risk, but there are few studies concerning eumenorrheic, premenopausal women. The purpose of this systematic review is to evaluate the most current research on this topic.

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**Keywords:** Physical Activity, Breast Cancer, Estrogen Metabolism, Premenopausal Women
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To my grandmother: You set the example of an extraordinary strong woman.

To my parents, family and friends: Thank you for helping me to succeed and for supporting me with encouragement, study snacks, couches to crash on, and good cheer through the best and the worst of this adventure. Congratulations to my new colleagues, it has been an honor working with you.
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List of Abbreviations

2-OHE1........................................................................................................2-hydroxyestrone
16α-OHE1..............................................................................................16α-hydroxyestrone
BMI...........................................................................................................Body Mass Index
DHQ.........................................................................................................Diet History Questionnaire
GYN..........................................................................................................Gynecological
LDL..........................................................................................Low Density Lipoprotein
MET ........................................................................................................... Metabolic Equivalent
PAR-Q........................................................................................................Physical Activity Readiness Questionnaire
RPE........................................................................................................Rate of Perceived Exertion
VO2max.................................................................................................. Maximal Oxygen Consumption
WHLP.....................................................................................................Women’s Healthy Lifestyle Project
WHR........................................................................................................Waist-to-Hip Ratio
The Effects of Physical Exercise on Selected Markers of Estrogen Metabolism Linked to Breast Cancer Risk in Premenopausal Women

BACKGROUND

Physical activity is linked to breast cancer risk reduction in women, yet the mechanism remains largely unknown. Possible causes for this association have been hypothesized to include change in endogenous estrogen production, estrogen metabolism, circulating concentrations of peptide hormones and growth factors, obesity, central adiposity, and immune function. Other factors considered in the potential for breast cancer risk are body fat percentage, obesity, and body fat distribution, which might be influenced by physical activity.

The International Agency for Research on Cancer has identified body fat and physical inactivity as the most important avoidable cause of breast cancer. There is a great deal of evidence demonstrating this important link. Friedenreich et al found a 30-40% decrease in breast cancer incidence among women who are physically active. Physical activity has been found to alter the level of sex steroids in both premenopausal and postmenopausal women.

Epidemiological studies show breast cancer risk is hormonally mediated. Friedenreich et al conducted investigations and concluded that association of exercise with breast cancer risk might be mediated by changing estrogens, estrogen metabolism, and metabolic factors such as reduction of excess body weight or body fat. Lifestyle factors such as diet, weight loss, smoking, body fat, and physical activity can modify estrogen metabolism.

A cumulative lifetime exposure to estrogen has been associated with a higher breast cancer risk. Friedenreich et al hypothesized that physical activity might reduce breast cancer risk by lowering resting levels of estradiol and progesterone and increasing levels of sex hormone binding globulin. Estrogen metabolites, which result from the hydroxylation of the parent estrogens estradiol
and estrone, are implicated in the subtle link between estrogen and its proposed biological conditions like estrogen dependent cancers and osteoporosis.\textsuperscript{22,23}

**Estrogen Metabolism**

Estrone and estradiol are synthesized in the body from the androgenic precursors androstenedione and testosterone.\textsuperscript{18} These hormones are synthesized in the liver by the cytochrome P-450 enzyme aromatase.\textsuperscript{18} This reaction occurs in granulose cells, adipose cells, and placenta.\textsuperscript{18} These metabolites are further broken down by the body from inactive estrogen to the chemically active and unstable byproducts. The ovaries produce 17β-estradiol, which is the main source of estrogen for premenopausal women.\textsuperscript{24} The enzymes in mammary cells convert estradiol to estrone, which can be hydroxylated at positions 2 and 16α in their molecular structure.\textsuperscript{24}

Estrogen is metabolized by two main pathways. Endogenous estrogen in the body is converted to the more active estradiol, which is then oxidized to estrone.\textsuperscript{18} This estrone is hydroxylated to 2-hydroxyestrone (2-OHE1) or 16α-hydroxyestrone (16-OHE1)\textsuperscript{25,26} through competitive pathways.\textsuperscript{2} These pathways are irreversible, meaning that the hydroxylation cannot be reversed to produce the stable estrogen molecule once hydroxylation has occurred.\textsuperscript{18} The 2-hydroxylation pathway is three to four times greater than the 16α-hydroxlation pathway.\textsuperscript{25-27} There are other estrogen metabolites circulating in the body, including 4-hydroxyestrone and 2-methoxy-estradiol.\textsuperscript{27,28} These metabolites are still being researched, but there are much smaller levels of these metabolites in circulation.\textsuperscript{29}

The 16α-hydroxyestrone metabolite pathway for estrogen is considered estrogenic and may cause oxidative damage to DNA.\textsuperscript{30} This metabolite demonstrates estrogenic properties through covalent bonding with the estrogen receptor and stimulation of cell proliferation.\textsuperscript{31} These factors have been found to increase breast cancer risk.\textsuperscript{19,22} This metabolite is increased in breast cancer patients and women at high risk for breast cancer.\textsuperscript{32} Animal studies show this metabolite proliferates selectively in mouse mammary epithelial cells.\textsuperscript{25,32}
In contrast, the 2-hydroxyestrone metabolism pathway is considered anti-estrogenic.\(^2\) 2-OHE1 is a weak estrogen because of rapid methylation, a rapid clearance rate, and weak binding affinity for the estrogen receptor, and the anti-proliferative effect on mammary cells.\(^27\) This pathway has a protective effect on estrogen metabolism and is associated with a reduced breast cancer risk, and a greater rate of breast cancer survival.\(^5\) Results from animal studies show clear evidence of protection against mammary tumors;\(^23,32\) it also decreases unscheduled DNA synthesis, and suppresses proliferation of human breast cancer cells.\(^25,31\)

Friedenreich et al was the first to hypothesize that physical activity might reduce those estrogen metabolite levels that are implicated in breast cancer risk.\(^1\) However, the level of physical activity in many previous studies has not been significant enough to decrease the absolute amounts of circulating estrogens.\(^33\) Snow et al found changes in concentrations of sex steroids among elite athletes who experienced menstrual dysfunction, but further studies failed to replicate this in normally active young women.\(^17,33\) From there, follow-up investigations tended to include exercise matched with concurrent calorie restriction because of the hypothesis that exercise alone is not enough to change the central control of the reproductive axis and produce a hypothalamic/pituitary response.\(^33\)

Another theory that sparked further studies was the potential for a change in the metabolite ratio to favor the anti-estrogenic 2-hydroxyestrone pathway, even if the total concentration of the estrogen metabolites remained unchanged.\(^33\) Previous research shows intense exercise increases 2-OHE1 formation but there is little research on less extreme exercise and its effect on estrogen metabolism.\(^3\) Previous measures of estrogen metabolites relied on radiolabeled tracers and gas chromatography-mass spectroscopy.\(^18\) Newer methods of measuring estrogen metabolite ratios through simple urine samples have lead to increases in research potential.

The 2:16 estrogen metabolite ratio may also be influenced by diet, physical activity levels, and smoking.\(^11,17\) Atkinson et al found that changes in intra-abdominal fat were associated with changes in
the 2:16 estrogen metabolite ratio.\textsuperscript{20} Interest in the newest idea that the 2:16 estrogen metabolite ratio might affect the breast cancer risk of women sparked further breast cancer prevention research.

**Limitations of Current Research**

Current research is conflicting in regards to the potential effect of this estrogen metabolite ratio and the potential for breast cancer risk reduction related to physical activity. Some studies show a positive link,\textsuperscript{15,16,22,34} but not all,\textsuperscript{12,26} and most prospective cohort investigations show only a non-significant risk reduction.\textsuperscript{30} Many investigations are limited to only elite athletes.\textsuperscript{35,36,17,37} Furthermore, many investigations include participants with menstrual dysfunction related to physical activity, use only cross-sectional analyses, or rely on only self-reports of physical activity without conducting a controlled intervention.\textsuperscript{2} The reduction in sex steroid hormones related to physical activity might be confounded by or related to menstrual dysfunction.\textsuperscript{17,37}

**Purpose of the Study**

The purpose of this investigation was to conduct a systematic literature review to determine the effects of physical activity on estrogen metabolite ratios in premenopausal women. Specifically, this investigation was conducted to determine if the 2:16 estrogen metabolite ratio is increased through physical activity to favor the anti-estrogenic estrogen metabolite associated with reduced breast cancer risk.

**METHODS**

**Search Protocol**

A systematic review of the current medical literature was conducted using four main literature review databases. The databases included in the search were MEDLINE, CINAHL, All EBM Reviews, and EPPI-Centre Database of Health Promotion Research (Bibliomap). Search terms were mapped to
established, standardized medical subject headings (MeSH) when made available by the search engine. For example, when searching the MEDLINE database, search terms were mapped to the MeSH terms “breast neoplasms,” “estrogens,” “exercise,” and “premenopause.” Terms used in searching all databases included “physical activity,” “2-hydroxyestrone,” “biological markers,” “estrogen metabolism,” “estrogens,” “hydroxyestrone,” and “estrogens, catechol.” Only randomized controlled trials, and prospective and retrospective cohort studies were included in this review.

All articles referenced in this systematic literature review were critically appraised and ranked on validity measures using a validity measure scoring system relevant to this subject matter (See Appendix). The summary of the critical appraisals of the included articles is listed in Table 1.

**Inclusion and Exclusion Criteria**

This search was limited to randomized controlled trials, and prospective and retrospective cohort studies that were conducted on a specific population and measuring explicit outcomes. Case-control studies have been excluded. The population considered for this review included premenopausal women who did not have a current diagnosis or history of breast cancer. The outcomes of the included studies were measured as a change in the ratio of estrogen metabolites linked to breast cancer risk. Specifically, only studies that measured the ratio of 2-hydroxyestrone to 16α-hydroxyestrone were included in this systematic review. Studies were excluded if the population included only postmenopausal women, if the population had current incidence or history of breast cancer, and if the outcomes of the studies were not measured as a change in the estrogen metabolite ratio. Articles were included in the analysis if the outcomes measured also included various estrogen metabolites, such as 4-hydroxycatecholestrone, or if other variables such as BMI or body composition were included in analysis. However, these other factors were not analyzed for the purposes of this systematic review.
RESULTS

A total of 39 records were identified through database searching according to medical subject headings. A thorough search through the references of identified articles revealed 104 additional articles for review. After duplicates were removed, 49 articles were screened for inclusion criteria, and 31 were removed because they were not randomized controlled trials or cohort studies. The full-text articles meeting this criteria were assessed for eligibility and 24 were excluded because the subjects were either postmenopausal, had breast cancer diagnoses, or the results were not measured as a change in the estrogen metabolite ratio. A total of six articles meeting the inclusion criteria were analyzed for the purposes of this systematic literature review. Only two randomized controlled trials concerning the population in question were discovered, and the remaining studies included in the review are cohort studies. Please see Table 2 for complete information regarding specific inclusion and exclusion criteria of the selected studies.

Campbell et al 2007

A randomized controlled trial conducted by Campbell et al, 2007\(^{30}\) attempted to determine the effects of a 12-week aerobic exercise training program on estrogen metabolites in sedentary or recreationally active premenopausal women. Caucasian females who were sedentary or recreationally active were included in this study. Recreational activity levels were defined as less than 20 minutes per day, three times per week in the past six months, with no formal aerobic training during the past year. Fitness levels of participants were measured using standardized VO2max measurements, and participants with measurements greater than 40 mL/kg/min were excluded. Other inclusion criteria were age between 20-35 years, regular menstrual cycles, and body mass index (BMI) 18-29.9kg/m\(^2\). Regular menstrual cycles for the purposes of this investigation consisted of cycle length of 24-36 days, with menstrual cycles for at least ten out of the past twelve months.\(^{30}\)
Participants in this study were excluded if they had used hormonal contraception or tobacco products in the past 12 months. Participants were also excluded from the study if they were vegetarian, if they had any metabolic health conditions such as thyroid disorders, diabetes, or liver disorders. Finally, participants were excluded if they were currently taking any medications that can interfere with hormonal pathways such as antidepressants or antibiotics, or if they had any pre-existing musculoskeletal conditions which prevented them from participating in an exercise program.  

Initial measures of height, weight, BMI, and waist-to-hip ratio were completed for each individual. Each participant filled out a three day diet log prior to participation, and calculations were made to ensure there was no baseline difference in amount of cruciferous vegetables or soy products linked to alterations in estrogen metabolism. Additionally, aerobic fitness was measured with a VO2max test using an incremental graded exercise stationary bike, with the criteria for reaching VO2max set as a plateau in oxygen consumption with increasing power output and/or respiratory exchange ratio of >1.1.  

The 17 participants randomized to the intervention group participated in a 12 week graded and individualized exercise program. Participants attended supervised exercise sessions 3-4 days per week. Exercise intensity was modified based on a six week repeat VO2max test. Meanwhile, the 15 participants randomly assigned to the control group were advised to maintain their normal level of activity and dietary intake. After the conclusion of the clinical trial the participants in the control group were given individualized guidance for starting their own exercise program, along with access to the fitness facility for four weeks.  

First morning urine samples were collected for all individuals in this investigation during the luteal phase of their menstrual cycles, standardized at day 20-22 since the self-reported first day of their last menstrual period. First morning fasting saliva samples were collected on days 19-22 of the menstrual cycles of all participants to confirm mid-luteal progesterone surge.
Fourteen out of seventeen members of the intervention group completed at least 80% of the supervised exercise sessions. The exercise group increased their aerobic capacity measured by VO2max, while the control group decreased their measured aerobic fitness (+4.6mL/kg/min, and -1.0 mL/kg/min, respectively). There was no significant change found in either group for body weight, BMI, or waist–to-hip ratio (WHR). The exercise group lost a mean of 1.2kg fat mass and gained a mean of 0.9kg lean mass. The baseline 2:16 ratio was significantly associated with percentage of body fat. Those with the highest percentage of body fat had significantly lower 2:16 ratios, meaning the concentration of 2-hydroxyestrone was relatively low in comparison to those individuals with a lower percentage of body fat. There was no change in the 2:16 ratio after intervention among the exercise group. There was no change in 2:16 ratio from baseline in either group. The improvement in aerobic fitness capacity did improve the 2:16 ratio. There was a positive association that approached significance between the percentage of lean body mass and 2:16 ratio.30

The end result of this investigation was that 12 weeks of aerobic exercise training was great enough to bring about an improvement in VO2max and modest body composition changes, yet still had no significant effects on estrogen metabolism enough to alter the ratio of 2-OHE1 to 16α-OHE1. This study concluded that potentially exercise and physical activity interventions may not have significant effects on estrogen metabolism in premenopausal women.30

Pasagian-Macaulay et al

Pasagian-Macaulay et al18 endeavored to assess the reliability of measuring estrogen metabolites in urine, and to measure the relationship of body weight, dietary fat and exercise to urinary estrogen metabolite ratios in premenopausal women. Participants in this study were 179 premenopausal women aged 44-50. The participants were pooled from the Women’s Healthy Lifestyle Project (WHLP)18. Participants were not currently taking any hormonal replacement or contraceptives.
All participants had a BMI of 20-34 kg/m² and were not enrolled in any weight reduction programs during the previous four months. 

Participants were excluded from the investigation if they had experienced two months of amenorrhea during the previous six months. Potential participants were also excluded if they had been hospitalized at a psychiatric facility within the previous year, or had a previous history of cancer within the past five years. No participants were accepted if they were currently using antihypertensive medications, had an elevated LDL or total cholesterol, or had an elevated fasting blood glucose reading above 140. Additionally, participants were excluded if they drank more than five alcoholic beverages per day, or if they had had a hysterectomy or bilateral oophorectomy.

A standardized Paffenberger physical activity questionnaire was given to all participants at the start of the trial to assess current leisure physical activity levels. The participants randomly assigned to the intervention group attended a 20 week program lead by trained nutritional and behavioral interventionists. The goals of the program were to reduce fat intake to less than 25% of total calories, reduce saturated fat intake to less than seven percent of total calories, reduce cholesterol intake to less than 100mg per day, and to increase moderate physical activity in the form of walking to expend about 20% of the daily total of calories. The intervention group attended 15 sessions with training on diet and exercise to achieve modest weight loss. The weight loss goals for those with a normal BMI (<24.44) were set at five pounds total over the six month time period of this investigation. Those with a BMI 24.45-26.44 were assigned a ten pound weight loss goal, and those with a BMI higher than 26.44 were given a 15 pound weight loss goal. All participants in the intervention group followed a 1300 or 1500 calorie per day meal plan for four weeks, and then followed their own self-modified diet with dietary logs for the remainder of the intervention.

The participants in the intervention group were all encouraged to increase physical activity starting at week three of the intervention. Participants were given instructions to walk ten miles per week over three to five days, with a goal of expending 1500 calories per week. Those participants who
already were expending 1500 calories per week through modest activity were encouraged to maintain their current level of physical activity. All physical activity was self-regulated and self-reported. There was no formal supervision of the exercise portion of the intervention.\textsuperscript{18}

Both groups were evaluated in the clinic at baseline and at six months. First morning urine samples were collected individually after a ten hour fasting period at baseline and at six months. Vitamin C was added to the samples as a preservative. All samples were stored on dry ice for 48 hours while shipping to the testing facility. Subjects were separated into three tertiles based on their estrogen metabolite ratios at baseline measurements. The low estrogen metabolite ratios were 0.5-1.75, the medium metabolite ratios were 1.76-2.47, and the high estrogen metabolite ratios were greater than 2.47. There was no attempt made by the authors of this investigation to standardize the urine samples according to the phase of the menstrual cycle.\textsuperscript{18}

There was a significant increase in the estrogen metabolite ratio 2:16 for both intervention and control groups, but no statistically significant difference between both groups for change in ratio. That is, both groups experienced an increase in ratio of estrogen metabolites, but there was no greater change in the exercise intervention group as compared to the control group. There was no significant relationship between any of the baseline risk factors and the baseline 2:16 ratio in either group. The intervention group improved their physical fitness, as measured by the standards set by the authors of this investigation. The intervention group lost an average of ten pounds and increased their total exercise amount by 400 calories per week. The increases in weight, BMI, and waist-to-hip ratio in the control group were all significantly correlated with the changes in the 2:16 ratio, but this relationship was not found in the intervention group.\textsuperscript{18}

The authors of this study hypothesized that perhaps their subjects lost weight but didn’t sufficiently deplete fat stores to increase the 2-hydroxylation pathway of estrogen. The authors suggested that perhaps only a combination of significant increase in exercise and decrease in total calories and fat will alter estrogen metabolism enough to result in a positive change in the 2:16
estrogen metabolite ratio. Another theory brought forth by the authors of this investigation was that this ratio might be more determined by genetics than the authors had originally hypothesized.\(^{18}\)

There were three hypotheses produced by the authors of this study to determine the results they found. They hypothesized that the ratio of specific metabolites might have less relation to breast cancer risk than the total production of estrogen. Second, the metabolism of estrogen might be more determined by environmental factors, such as phytoestrogens in plants, than by level of physical activity. Finally, the metabolites of estrogen that are established risk factors for breast cancer might be more determined by genetics and environmental factors than originally hypothesized by the authors of the current study. The authors rationalized that postmenopausal breast cancer risk is higher because postmenopausal estrogen is primarily metabolized from the aromatization of andostenedione in fat tissue, and is no longer produced from the ovaries. Due to this factor, the authors reasoned that obesity is a significant determinant of estrogen levels in postmenopausal women, rather than in premenopausal women.\(^{18}\)

The weight difference between groups in this study was significant, but did not produce changes in 2-hydroxylation of estrogen. The authors suggested that postmenopausal women metabolize estrogen more along the 16α-hydroxylation pathway, so a more estrogenic shift in postmenopausal women can perhaps reflect a greater effect from physical activity levels than in pre-menopausal women, who already metabolize estrogen primarily along the 2-hydroxylation estrogen pathway.\(^{18}\)

**Westerlind et al**

Westerlind et al\(^{33}\) sought to discover whether physical activity and/or energy deficiency increased the 2:16 ratio, and whether or not this is a factor in the reduction in breast cancer risk correlated with physical activity. Also, the authors of this clinical trial attempted to determine the effect of moderately intense exercise training combined with calorie restriction on 2:16 ratio of
estrogen metabolism. The measurement of an increase in physical activity was determined to be the loss of body weight and body fat, and an increase in VO2max.  

The authors of this study set specific inclusion criteria for participants. All participants had to be free of any serious medical conditions, without current depression or eating disorders. Participants were between the ages of 25 and 40, and weighed 50-90 kg. Members of this study were required to have a body fat percentage between 15-45%, and BMI 18-35. Participants were non-smokers, who were not currently taking any anti-depressants or antibiotics, had no significant weight changes during the past 12 months, and exercised less than one hour per week. All participants had to have a gynecological age of at least 13 years, were not using any hormonal contraception during the past six months, had regular menstrual cycles, and were free of any musculoskeletal conditions that might limit participation in any physical exercise.

A total of 31 subjects began a four month intervention of moderate aerobic exercise and calorie restriction. Twenty-four participants completed the entire study. Those participants who dropped out of the investigation were those in the group who had significantly higher percentages of body fat and BMIs. Participants were measured for one complete baseline menstrual cycle, and four subsequent menstrual cycles during the intervention phase. All subjects attended supervised informational sessions designed to give participants the tools to increase their physical energy expenditure by twenty percent. In addition, all participants were encouraged to decrease total caloric intake by 20-35%. Daily menstrual records were kept by each participant, with daily urine collections to verify hormone levels. Urine was analyzed for estrogen metabolites and 2:16 ratios during the mid-folicular and mid-luteal cycles of each participant individually.

Baseline depression and eating disorder inventories were taken on all participants, and subjects were compared from baseline complete blood counts, chemistries, and endocrine screening. Diet records were kept for three to four days and submitted by all subjects. Baseline aerobic fitness capacity was measured using VO2max recordings on a standardized stationary bicycle. Body composition was
measured with hydrostatic weighing. Menstrual status of each participant was measured at baseline, and then measured daily during the control menstrual cycle, and each subsequent intervention cycle.\textsuperscript{33}

Caloric restriction was performed by each subject individually. Participants kept self-reported diet logs and met with a dietician every two weeks. Diet was adjusted individually if there was no recorded weight loss, and measurements of cruciferous vegetable and soy intake were monitored to ensure there was no significant difference among participants.\textsuperscript{33}

All participants attended supervised workout sessions four times per week with personal trainers. Each workout lasted 40-90 minutes, and workouts were personalized with the end goal of achieving 60-90\% of maximal heart rate. There was an average of 96\% attendance of the exercise sessions, with an average of 3.6 workouts logged by participants per week. Caloric expenditure was individually measured and standardized to achieve 20\% of each participant’s caloric intake. Fist void urine samples were brought in daily on ice from each participant. Each urine sample was preserved with boric acid, refrigerated for no more than four hours before processing, and shipped on dry ice to the standardized testing facility. The 2:16 ratio of estrogen metabolites was normalized to creatinine concentration in the urine samples.\textsuperscript{33}

The baseline measurement of 2-hydroxyestrone metabolites was inversely correlated with baseline BMI. Over the course of the study, significant changes in weight, BMI, body fat, VO2max, and caloric intake were achieved, but there was no statistically significant change in lean body mass percentage. There was no difference in the ratio of 2-hydroxyestrone metabolism to 16α-hydroxyestrone metabolism between the follicular and luteal phases, although the total concentrations of both estrogen metabolites were found to be significantly higher during the luteal phases of each participant. The only statistically significant change in estrogen metabolite concentration found, was an increase in 16α-hydroxyestrone metabolism during the luteal phase, and a nearly significant increase in 2-hydroxyestrone metabolite concentration also during the luteal phase.\textsuperscript{33}
The authors of this investigation found an association between the change in the ratio of 2:16 metabolism of estrogen and the baseline ratio of 2:16. This development led the authors to hypothesize that some women are considered responders, while others are non-responders. That is, some women respond to exercise interventions with an increase in the 2:16 ratio of estrogen metabolism, while other women do not. Subjects were divided into Tertiles based on this theory. Tertile 1 had an average baseline ratio of 0.91. Subjects in Tertile 2 had an average 2:16 ratio of 1.97, and participants in Tertile 3 had the highest average baseline ratio of 2.95. There was no significant difference between Tertiles as far as weight, fat, fitness, percent fat loss, calories consumed, or menstrual cycle length. The only significant difference found was that exercise intensity was lower Tertile 2. The women with the lowest baseline ratio of 2:16 metabolism experienced the greatest increase in 2:16 ratio during intervention. This higher increase was found in both categories of percentage increase, and absolute increase in ratio.  

The authors of this investigation believed that it was unlikely that exercise intensity was associated with the change in 2:16 ratio. Since the participants were involved in exercise, calorie restriction, and weight loss simultaneously, it is impossible to determine the specific cause of the changes in metabolite ratios. Those participants in the lowest Tertile of baseline ratio did not lose more weight or fat or become more aerobically fit. Therefore the authors hypothesized that the favorable change in estrogen metabolites associated with a decrease in breast cancer risk is not determined by initial body fat or body weight, or by the magnitude of change in body composition from exercise. Rather, the change might be most determined by the woman’s initial estrogen-metabolism profile. The investigators developed the theory that women with a higher initial ratio of 2:16 metabolism might already be at a lower risk for developing breast cancer. Accordingly, these women might not experience a significant change in estrogen metabolite concentration due to physical activity. The authors suggested that estrogen metabolite profiles of women could potentially be used to identify women at higher risk for breast cancer if they have lower ratios of 2:16 metabolism of estrogen, and
these particular women could be identified as a population who might benefit most from targeted intervention designed to increase physical activity in order to increase the anti-estrogenic biochemical metabolism.33

Schmitz et al

Schmitz et al38 investigated the effects of 15 weeks of aerobic exercise training on oxidative stress, estrogen metabolites, and body composition in 15 premenopausal, eumenorrheic women. All women included in this study were between 18-25 years old and experienced regular menstrual cycles. All participants were nulliparous with intact ovaries and uterus. The participants were sedentary, which was defined as less than two moderate intensity exercise sessions per week for the past six months. Participants had BMI 18-40 and consumed less than seven alcoholic beverages per week. Participants were excluded if they had used hormonal contraception within the past year or were pregnant in the past six months. Participants were also excluded if they had any gynecological disorders such as fibroids, endometriosis, or polycystic ovarian syndrome. Additionally, women were excluded from the study if they had uncontrolled hypertension, had used tobacco products during the previous month, or had any physical conditions limiting their participation in an exercise intervention program.38

All participants in this investigation had clinic visits at baseline and post-intervention during the follicular phase of their menstrual cycle. The clinic visits all took place after a 12 hour fast, and at least 48 hours after completion of any exercise. There was a 54% dropout rate during this investigation. Only 15 participants of 28 completed this study.38

Baseline body composition was measured with dual energy x-ray absorptiometry, and baseline fitness was assessed by maximum heart rate, based on participants reaching 80% of their age-based calculated maximum heart rate. All participants also completed baseline Diet History Questionnaires to measure their dietary intake at the onset of the investigation. First void urine samples were collected on three consecutive days. All samples were preserved with sodium azide and normalized to creatinine
concentration. All subjects started exercise intervention on the eleventh day of their self-reported menstrual cycle and stopped on the fifth day of their menstrual cycle 3-4 cycles later.\textsuperscript{38}

All participants attended five weekly training sessions that included 30 minutes of physical activity with a warm up and cool down. The goal for all participants was to achieve 70-85\% of their maximum heart rate, with heart rate goals increased weekly. Workouts were supervised once per week, and the remainder of physical exercise was self-recorded.\textsuperscript{38}

There was an 87.5\% adherence rate to the exercise program, with a target level of heart rate achieved 95.3\% of the time. There was no statistically significant change in absolute concentrations of 2-hydroxyestrone or 16α-hydroxyestrone, or in the 2:16 ratio from baseline to intervention. A marker of lipid peroxidation, F2-isoprostanes, declined slightly from the course of baseline through intervention. There were also significant decreases in body weight, BMI and body fat percentage. This caused the investigators to believe that some of the radicals that lead to the peroxidation of lipids may damage DNA. They theorized that exercise has the potential to significantly alter the formation of free radicals and lipid peroxidation, and this may be influential in future efforts towards cancer prevention.

The authors concluded that exercise decreases the potential for developing certain types of cancers, but not because of a change in 2:16 ratio of estrogen metabolism. The free radicals associated with lipid peroxidation were thought to be an area for potential future research in the field of cancer prevention. The authors of this article theorized that a woman needs enough of an energy deficit to cause weight loss and produce anovulation in order to change estrogen metabolism, and those results were not found in this study. The researchers suggested future research should concentrate on luteal phase estrogen metabolism, as this phase measured the highest total estrogen concentrations.\textsuperscript{38}

\textbf{Bentz et al}

Bentz et al\textsuperscript{34} sought to determine whether higher levels of physical activity might be correlated with higher levels of 2-hydroxyestrone metabolites and higher 2:16 ratios. The authors also desired to
determine if the 2:16 estrogen metabolite ratio might be a biomarker for breast cancer risk reduction that can then be incorporated into exercise programs based on a specific intensity and duration of exercise.34

Seventy-seven women in this study met the specific inclusion criteria for participation. All volunteers were eumenorrheic over the previous year, were non-smokers, were not taking hormonal contraceptives, and had a BMI 18-30. Women were excluded from this study if they were vegetarian, pregnant or lactating, or had any metabolic disorders such as thyroid imbalance or diabetes. Additionally, all African Americans were excluded from this study because of the prevalence for polymorphisms in the gene that produces the 2-hydroxylating enzyme.34

Urine samples were collected during the self-reported luteal phase of participants. Diet was self-recorded on two weekdays and one weekend day for two weeks prior to specimen collection. Physical activity logs were self-maintained by participants for two weeks prior to urine sampling. Physical activity reported on the logs included household work, leisure activity, occupational activity, and sport-related physical activity. All reports of activity were analyzed using Borg’s modified ratings of perceived exertion (RPE) to calculate standardized metabolic equivalent (MET) values and total MET hours of physical activity per week.34

First morning urine samples were preserved with ascorbic acid, refrigerated for no more than four hours prior to handling, transported on ice to the standardized treatment facility, and normalized to each individual’s urine creatinine concentration. Participants were separated into overweight (BMI greater than 25) and normal weight (BMI 25 or less) groups to examine the relationship between physical activity and to rule out BMI as a possible confounding factor. Also, amounts of brassica vegetable and supplement intake were measured to rule out differences between groups. No significant differences between groups were noted.34

The amount of self-reported MET hours per day ranged from zero to 14.31 for all participants. Significant differences were found between concentrations of 2-hydroxyestrone and 2:16 ratios related
to MET hours of physical activity. Age and BMI were statistically controlled for, with no effect of possible peri-menopause found. The normal BMI group did not show significant relationships between MET hours of physical activity and estrogen metabolites when analyzed based on BMI alone. The overweight group did demonstrate a significant relationship between MET hours of physical activity and concentrations of both 2-hydroxyestrone and 2:16 ratio of metabolites. When participants in the overweight BMI group were studied individually, 11 out of 19 identified themselves as highly competitive and reported exercising 5-7 days per week. The authors of this investigation concluded that stratifying participants based on BMI alone is not an accurate measure of body composition in athletes. The participants in the higher BMI group in this study most likely did not have high body fat percentages, since the majority of them were competitive athletes.

No relationship was found between physical activity and BMI. There was also no significant relationship between 16α-hydroxyestrone concentration levels and levels of self-reported physical activity. The authors of this investigation separated participants into quartiles based on reported MET hours per week of physical activity. There was no statistically significant difference between quartiles for 2:16 estrogen metabolite ratio, but the difference between groups approached significance, with the most physically active group measured to have the highest 2:16 metabolite ratio. The frequency of physical activity was significantly different between the highest and lowest quartiles, along with the baseline 2:16 ratio and baseline 2-hydroxyestrone concentration.

At the conclusion of this investigation, authors determined that sex hormones might be modulated by physical activity to favor the weak estrogen, 2-hydroxyestrone. This change in metabolism might be a potential mechanism for the breast cancer risk reduction correlated to physical activity. In light of evidence that body fat percentage is correlated with estrogen metabolism and the findings of an overweight BMI group, which turned out to be comprised of mostly athletes, the authors concluded that body fat percentage might indeed be a confounding factor in the measurement of 2-hydroxylation of estrogens. Women in this study who were most active had the highest baseline 2:16
ratio. The authors concluded that exercise intensity beyond moderate might be required in order to significantly change estrogen metabolism.34

**Campbell et al 2005**

Campbell et al, 2005² investigated the association between aerobic fitness levels and 2:16 estrogen metabolite ratio, and total concentrations of each estrogen metabolite in premenopausal women. Their hypothesis was that premenopausal women with higher aerobic fitness levels would have higher concentrations of the anti-estrogenic 2-hydroxyestrone, and lower concentrations of the estrogenic 16α-hyrdoxyestrone, and therefore a higher 2:16 ratio.²

Caucasian women aged 20-42 years old were included in this study. Participants were required to have regular menses and a BMI 18-24. Volunteers were excluded from participation if they were smokers, had taken hormonal contraception in the previous six months, if they were vegetarian, had any endocrine disorders, were currently taking antibiotics or antidepressants, or had any physical conditions limiting exercise participation.²

This investigation was carried out in an extreme group split design. The participants all filled out Godin Leisure Time Exercise Questionnaires²¹ and were separated into groups on either end of the physical activity spectrum. Those who reported strenuous exercise 3-5 days per week for the previous six months were selected for one group, and those who reported little or no physical activity were selected for the other group. All volunteers also completed a PAR-Q Physical Activity Readiness Questionnaire, and then were subjected to a graded exercise test with indirect calimetry using VO2max to determine baseline physical fitness level. The high fitness group had VO2max scores greater than 48ml/kg/min, while those in the average fitness group had scores of less than 40ml/kg/min. Baseline measures of the sum of skin folds were substituted for the percentage of body fat, and the BMI of each participant was also measured at the start of the trial. The diet of each individual was assessed over the previous 12 months using a standardized Diet History Questionnaire.²¹²
Two first morning urine samples were collected from each participant after a ten hour fast between days 4-6 and 20-22 of the same menstrual cycle of each woman. No physical activity was allowed 24 hours prior to urine sampling. The urine samples were stored on ice and processed within four hours, with ascorbic acid added as a preservative. The menstrual status of each individual was determined from saliva samples collected from day 12 forward in the menstrual cycle.²

A total of 13 out of the 18 members in the average fitness group completed the study, while 17 out of the original 18 members of the high fitness group completed the study. The members of the high fitness group reported about 368 minutes of physical activity per week, compared to about 64 average minutes of physical activity reported from the average fitness group. There was no significant difference between groups in categories of age, body weight or BMI. The average fitness group had a higher percentage of body fat, measured by sum of skin folds. There was no significant difference found between the groups as far as the total concentrations of either estrogen metabolite, or the 2:16 ratio. The high fitness group showed a trend towards higher luteal 2:16 ratio, although no results were statistically significant. The high fitness group consumed more calories, carbohydrates, fiber and vegetables than the average fitness group; however, these differences were not found to be statistically relevant to the changes in the 2:16 ratios. A higher BMI and body fat percentage was associated with a lower 2-hydroxyestrone concentration and a lower 2:16 ratio in further analysis after the conclusion of the trial. Also, the total concentration of the more estrogenic 16α-hydroxyestrone was found to be higher in those with higher BMI and percent body fat.²

The authors of this investigation theorized the potential reasons for the non-significant changes in estrogen metabolite ratios. The thresholds set to evaluate physical fitness using VO2max scores might not have been accurate. The investigators suggested that estrogen metabolism changes due to physical activity might only occur when moving from low to average fitness, rather than from average to high fitness. They theorized that physical activity might affect estrogen metabolism, but perhaps not because of aerobic fitness, which was the measure set by this study. Physical activity might have a
greater effect on sex steroids, circulating growth factors, or binding proteins than on the estrogen metabolite ratio. Alternatively, physical activity might instead affect body composition, immune function, or antioxidant defenses, which might alter breast cancer risk by changing estrogen metabolism. The authors concluded that these changes might not necessarily increase aerobic fitness levels.2

**DISCUSSION**

There was a great deal of variability across all six studies included in this review. One large difference between the articles was the inclusion and exclusion criteria. Some investigations sampled a very specific population based on extensive inclusion and exclusion criteria,30,2,33 while other studies were not as specific.18,38 Some studies included only athletic or moderately athletic individuals,18 some looked at only sedentary individuals,30,38 and some looked at both extremes.34 As Westerlind et al33 discovered, the baseline 2:16 ratios were important in determining the potential for change in those estrogen metabolites according to physical activity intervention. If there was a wide variation in baseline levels of physical activity between individuals, groups, and studies, then the results gathered from these investigations might be suspect. This difference in baseline characteristics of the populations under investigation might affect the results.

There were specific limitations in each study included in this review that warrant further discussion. The differences in populations investigated, interventions, and statistical analysis methods make comparisons between studies difficult. Many studies were limited by small sample size and lack of a control group for comparison.33,38,34,2 Also, there was little standardization between studies for menstrual cycle. Some investigations measured hormonal changes to determine stage in menstrual cycle,30,2,33 while others used only self reported measures,38,34 and others did not attempt to control for menstrual stage at all.18 Some studies prior to this review used older methods of estrogen metabolism measurement. All studies included in this review used newer solid-phase enzyme immunoassay to
determine estrogen metabolite concentrations. Finally, there were variations among studies in baseline physical measurements. Some investigations measured body composition with hydrostatic weighing or dual energy x-ray absorptiometry technology,\textsuperscript{33,38} while others simply used BMI as a substitute for measurement of body composition.\textsuperscript{30,2,34} As Bentz et al\textsuperscript{34} determined, BMI is not a valid measure of body composition in a lean athletic population. Table 3 demonstrates the specific limitations of each investigation based on selected criteria.

**Type of Investigation and Absence of Control Group**

Of the six studies included in this review, only two can be classified as randomized controlled trials.\textsuperscript{30,18} The remaining four investigations were classified as cohort or crossover studies, meaning that the same participants were measured at baseline and after intervention and served as their own controls.\textsuperscript{33,38,34,2} Campbell et al, 2007\textsuperscript{30} and Pasagian-Macaulay et al\textsuperscript{18} were the only authors to include a control group in their investigations, and they were both limited by the fact that they did not have a similar intervention for the control group. Campbell et al, 2007\textsuperscript{30} advised the control group to continue with their regular diet and exercise routines, which might introduce a bias, as the samples were not blinded to their assigned treatment group. Pasagian-Macaulay et al\textsuperscript{18} failed to mention the control group in their investigation, which leads the reader to assume there was no planned placebo intervention for the control group. The remaining authors failed to use a control group at all, although Campbell et al, 2005\textsuperscript{2} divided the participants into a highly physically fit group and an average physically fit group for comparison.

An additional factor in some but not all investigations was the addition of dietary changes to physical activity level changes. There were no dietary changes reported by Campbell et al, 2007\textsuperscript{30} or Bentz et al\textsuperscript{34}, while Pasagian-Macaulay et al\textsuperscript{18} and Westerlind et al\textsuperscript{33} included dietary restriction as a vital component of their interventions. Further, Bentz et al\textsuperscript{34} and Campbell et al, 2005\textsuperscript{2} included self-
reported dietary logs of participants in their calculations to control for possible confounding factors, while the remaining authors did not. 18,30,33,38

Baseline Characteristics

One interesting finding by Pasagian-Macaulay et al 18 is the association between baseline 2:16 ratio and the percent of change of that ratio based on physical activity. This study was the only one of the six included in this review that recorded baseline 2:16 ratio for the purposes of comparison to outcomes. It would be very interesting compare the baseline 2:16 ratios in relation to outcome ratios of each investigation, if this data had been included in the other studies.

Some studies limited the amount of baseline physical activity, while others did not. This potential difference in baseline physical fitness might affect results. In fact, this baseline difference was used to separate participants into high and average fitness groups in order to compare retrospective reports of physical activity in the study conducted by Campbell et al, 2005. 2 In this investigation, participants were separated into two groups based on self-reported physical activity of 3-5 days per week and VO2max scores greater than 48, or no reported physical activity and VO2max scores less than 40. 2 The more recent study by Campbell et al, 2007 30 limited physical activities to less than 20 minutes per day, three times per week, with VO2 max scores less than 40. Westerlind et al 33 limited participants to less than one hour of physical activity per week prior to the start of the trial, while Schmitz et al 38 limited participants to less than two hours per week. Physical activity levels prior to investigation were not limited by Pasagian-Macaulay et al 18 or Bentz et al. 34 Moreover, some studies used precise measures of physical fitness level at baseline such as VO2max, 2,30 or heart rate, 38 while others relied on self-reports of physical activity, 33 and still others did not address this possible confounding factor. 18,34

Another baseline physical characteristic that turned out to be valuable in the post-intervention calculations that was not standardized was body composition. Each study used varying measures of
body composition that are limited by precision and accuracy. Pasagian-Macaulay et al\textsuperscript{18} and Bentz et al\textsuperscript{34} only measured BMI at baseline. Campbell et al, 2007\textsuperscript{30} associated BMI and waist-to-hip ratio with body composition, while the earlier investigation authored by Campbell et al, 2005\textsuperscript{2} used the sum of skin folds as a measure of body composition. Finally, Westerlind et al\textsuperscript{33} used the highly specific hydrostatic weighing technique to measure body composition, and Schmitz et al\textsuperscript{38} used another highly specific measure of body composition, x-ray absorptiometry. Generalizations based on these various measures of body composition need to be assessed with caution, as the measurements have varying degrees of precision.

\textbf{Variations in Intervention}

There were differences among studies not only in intervention length, but in whether the intervention was supervised, workouts were standardized or individualized, if the exercise was self-reported, and if the intervention was prospective or retrospective. Bentz et al\textsuperscript{34} conducted a prospective intervention that was two weeks in duration, while Campbell et al, 2005\textsuperscript{2} ran a retrospective investigation spanning 12 months. The remaining prospective trials lasted in duration from 12 weeks,\textsuperscript{30} to 20 weeks.\textsuperscript{18} Workouts were fully supervised in the studies conducted by Campbell et al, 2007\textsuperscript{30} and Westerlind et al,\textsuperscript{33} partially supervised in the investigation by Schmitz et al,\textsuperscript{38} and self-reported in the studies by Pasagian-Macaulay et al,\textsuperscript{18} Bentz et al,\textsuperscript{34} and Campbell et al, 2005.\textsuperscript{2}

\textbf{Standardization of Samples}

The estrogen metabolite ratios in all studies included in this review were measured with the newer solid-phase enzyme immunoassay. All studies measured estrogen metabolite concentrations in urine samples that were self-collected by the participants and brought in to the laboratory within a certain amount of time. All investigations used preservatives to maintain the active estrogen metabolites. The similarities between studies end there. Some samples were taken during the luteal
phase, some during the follicular phase, and some were not standardized to menstrual phase

Some studies stratified estrogen concentrations to creatinine concentrations for better accuracy, while others failed to do so.

The urine samples obtained in the study conducted by Pasagian-Macaulay et al were not standardized to menstrual phase at all. Schmitz et al collected urine samples during the self-reported follicular phase, while Bentz et al collected urine samples during the self-reported luteal phase. The recent investigation of Campbell et al, measured luteal phase urine samples, verified by salivary hormone levels, while the previous study by Campbell, measured both luteal and follicular urine samples, also verified by salivary hormone measurements. Finally, Westerlind et al measured both follicular and luteal hormone levels and verified menstrual phase by daily urine hormone level concentrations. Varying results were found between the luteal and follicular phases, with higher absolute concentrations in the luteal phase. Therefore, samples not standardized to luteal phase of the menstrual cycle may not be an accurate measure of hormone concentrations.

Sample Size and Dropout Rate

Many of the investigations included in this review were limited by small sample size, high dropout rate, or both. The largest investigation was conducted by Pasagian-Macaulay et al, and included 84 participants in the intervention group and 90 in the control group. Bentz et al also had a larger population in comparison, with 77 total participants. Dropout rate was not addressed by either study. Schmitz et al experienced the highest dropout rate over the course of their investigation. Only 15 out of 28 total participants completed the study, which is a 54% dropout rate. In the latest investigation by Campbell et al, 14 out of the original 17 completed at least 80% of the intervention workouts, and 15 out of 15 participants assigned to the control group completed the study. Twenty-four of the original 31 participants in the study by Westerlind et al completed the trial.
Finally, in the earlier investigation lead by Campbell et al, 2005, 2 17 out of 18 in the high fitness group completed the investigation, while 13 out of 18 in the average fitness group completed the study.

**Aerobic Fitness Measurements**

The measures of physical fitness from baseline to the conclusion of the study differed among authors. This variation might lead readers to conclude that increases in physical fitness reported by the study might not be accurate. Physical fitness was not measured at the start or conclusion of the trial led by Bentz et al, 34 while it was only measured at baseline in the studies by Schmitz et al 38 and Campbell et al, 2005. 2  Variations in baseline measurements discussed above might also affect the results of these investigations. Physical fitness was measured only by change in weight and BMI by Pasagian-Macaulay et al, 18 while it was measured with standardized VO2max scores in the studies conducted by Campbell et al, 2007 30 and Westerlind et al, 33

**Race**

The final variation among these investigations that might affect the results of the studies and the ability of the reader to generalize these results is the selection of participants based on race. Some investigators limited their study populations to Caucasian females only, 2, 30 while others did not limit their study participants based on race at all. 18, 33, 38  Bentz et al 34 specifically excluded African American participants based on the prevalence for a genetic mutation in estrogen metabolism. If this genetic predisposition is verified by scientific investigation and prevalent in African American populations alone, then the inclusion of this population subgroup in other studies included in this review might affect their results.
Suggestions for Future Areas of Research

The authors of these six studies suggested many different avenues for potential future research, many of these suggestions based on limitations in their own investigations. One common agreement for an area of future research is to conduct further large, randomized controlled trials, with both short and long interventions on both diet and exercise, using objective measurements of physical activity recording that can easily be duplicated.

Other suggestions for areas of further investigation were the relationship between body composition and 2:16 ratio, the relationship between physical activity and other estrogen metabolites, and the effects of physical activity during other life stages. Based on their results, Westerlind et al suggested future research to concentrate on the baseline 2:16 measurements and the association with change in that ratio across intervention. Because Schmitz et al recorded the most statistical significance with results gathered during the luteal phase, the authors suggested future research should concentrate on the luteal phase in the menstrual cycle as the point when potential changes in estrogen metabolism can be best identified.

CONCLUSION

The results of these six investigations demonstrate that although there is an established breast cancer risk reduction for young women who are physically active, this risk reduction might not be achieved through the estrogen metabolism pathway. However, due to small sample sizes and lack of control groups or menstrual cycle standardization in many studies, it is nearly impossible to be able to generalize the results found in the studies to a larger population of women. There is potential that the effect physical activity has on estrogen metabolism was simply not great enough in the studies to be statistically significant due to variations in absolute hormone levels during menstrual cycles, or due to small sample sizes that did not present enough variety in baseline estrogen metabolite ratios.
Future research is needed first and foremost to determine if there is indeed an association
between breast cancer risk reduction and physical activity related to the change in estrogen metabolite
ratio to favor the anti-estrogenic metabolite 2-hydroxyestrone. To accomplish this, there needs to be a
number of randomized controlled trials that investigate the relationship of absolute concentrations of 2-
hydroxyestrone and 16α-hydroxyestrone, along with the ratio between the two metabolites. There are
several criteria that a future endeavor would need to meet in order to be more precise in treatment
effect and therefore results would be able to be generalized to a larger female population.

The estrogen metabolite concentrations that will be calculated from urine samples need to be
stratified to creatinine levels in urine to get an absolute concentration rather than a relative
concentration. The samples need to be standardized in their collection, preferably during the luteal
phase of the menstrual cycle, when the metabolites are all at their peak concentrations. If possible,
menstrual phase should be verified by measuring hormone levels, as many women have differing cycle
lengths. The method of collection of urine samples should be streamlined, and if possible, samples
should be taken in a laboratory facility, where temperature and time from sample collection to
processing can be standardized.

This ideal randomized controlled trial would have a population that included all ages, races,
levels of body composition, and levels of baseline physical activity. Ideally, the population studied
would be large enough to be generalized to the greater population of females. There would also need to
be a control group of participants with the same baseline characteristics, who undergo some sort of
placebo intervention simultaneously. The physical activity should be standardized and supervised
among participants, using objective measures of physical fitness and exertion. Other factors affecting
breast cancer risk, such as obesity, diet, and family history would need to be well controlled for. The
Physical Activity for Total Health (PATH) Study, designed to measure many of these criteria, is
currently underway.20 Perhaps results from this investigation will shed new light on the relationship
between physical activity and estrogen metabolism.
Other areas of future research need to concentrate on differing hypotheses about the potential causes of this association between exercise and reduction in breast cancer risk. There is a possibility that the risk reduction found in women who regularly exercise is limited to postmenopausal women.²⁰ The change in estrogen metabolism that occurs during menopause favors the more estrogenic 16α-hydroxyestrone metabolite, which in turn decreases the 2:16 metabolite ratio.⁴⁰ As Westerlind et al discovered,³³ the baseline ratios of 2:16 estrogen metabolism were inversely correlated with percent change in ratio during intervention. Since postmenopausal women have a lower baseline ratio than premenopausal women, perhaps this association is better seen in postmenopausal women.

Nevertheless, studies have shown a decrease in breast cancer risk in premenopausal women who are physically active,⁴,⁶ so more research is needed to determine the cause of this phenomenon.

Sex steroid concentrations, circulating growth factors and binding proteins are other areas that call for further investigation. There is evidence that these factors also affect breast cancer risk,²⁷,³¹ and future studies should concentrate on all potential factors with a controlled exercise intervention to determine which factor might potentially have the greatest effect on breast cancer risk.

Other potential causes for the change in estrogen metabolite ratio include body weight, BMI, body fat percentage, and lean body mass.²³,⁴² It is possible that the change in estrogen metabolism associated with reduced breast cancer risk is more related to body composition than to physical activity that changes body composition. Perhaps statistically significant changes were not found in these six interventions because there was not enough physical activity to produce the changes in body composition necessary to change the estrogen metabolite profile.³⁷ Previous studies show a greater breast cancer risk reduction in individuals who are active across their lifetime.⁸,⁹,¹⁴,⁴³ If this is the case, then physical activity, along with diet and lifestyle changes, might only be the method of achieving body composition changes that reduce breast cancer risk.
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### Table 1: Summary of Results of Selected Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Yr.</th>
<th>Patients/Population</th>
<th>Intervention</th>
<th>Comparison</th>
<th>Outcome</th>
<th>Study type</th>
<th>Validity Score</th>
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<tbody>
<tr>
<td>Campbell et al 2007</td>
<td>2007</td>
<td>Premenopausal females (see Table 2)</td>
<td>17 participants 12 weeks 3-4d/wk measured by VO2 max</td>
<td>15 participants Maintained normal activity</td>
<td>No change in 2/16 ratio in either group</td>
<td>RCT</td>
<td>8</td>
</tr>
<tr>
<td>Pasagian-Macaulay et al 1996</td>
<td>Premenopausal females (see Table 2)</td>
<td>84 participants 20 week intervention 5-15lb wt loss, reduce total fat, CHD, increase PA 20% of calories</td>
<td>90 participants Maintained normal activity level</td>
<td>Increased 2/16 ratio in both groups, no significant difference between groups</td>
<td>RCT</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Westerlind et al 2007</td>
<td>Premenopausal females (see Table 2)</td>
<td>31 participants 4 month intervention exercise combined with calorie restriction</td>
<td>All participants were compared to baseline measures of same</td>
<td>No statistically significant change in 2/16 ratio, but the lowest baseline 2/16 had the greatest change</td>
<td>Crossover</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Schmitz et al 2008</td>
<td>Premenopausal females (see Table 2)</td>
<td>15 total participants Exercise 5x/wk x12 weeks</td>
<td>Baseline measurements of same participants</td>
<td>No change in 2/16 ratio from baseline</td>
<td>Crossover</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Bentz et al 2005</td>
<td>Premenopausal females (see Table 2)</td>
<td>77 Participants Compared BMI, MET hrs/day, age,</td>
<td>Stratified into quadrants based on level of physical activity</td>
<td>Increased 2:16 ratio with increased physical activity BMI not reliable for athletes in study</td>
<td>Crossover</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Campbell et al 2005</td>
<td>Premenopausal females (see Table 2)</td>
<td>17 women Reported physical activity 3-5x/wk(368min/wk ave) Compared diet and PA</td>
<td>13 women Little or no exercise (average 64min/wk)</td>
<td>No change in 2:16 ratio between groups Increased 2:16 ratio and skin folds, BMI</td>
<td>Cohort</td>
<td>4</td>
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Table 2: Inclusion and Exclusion Criteria of Included Articles

<table>
<thead>
<tr>
<th>Study</th>
<th>Physical Activity</th>
<th>Age</th>
<th>BMI</th>
<th>Regular Menses</th>
<th>Hormonal Contraceptives</th>
<th>Tobacco</th>
<th>Medical conditions</th>
<th>Antidepressants or Antibiotics</th>
<th>Physical Limitations</th>
<th>Intact ovaries and uterus</th>
<th>Other Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al 2007&lt;sup&gt;30&lt;/sup&gt;</td>
<td>&lt;20min/day, 3x/week</td>
<td>20-35</td>
<td>18-29.9</td>
<td>24-36 day cycle, 10/12months</td>
<td>None x6 months</td>
<td>None x12 months</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>__</td>
</tr>
<tr>
<td>Pasagian-Macaulay et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>__</td>
<td>44-50</td>
<td>20-34</td>
<td>10/12 months</td>
<td>None</td>
<td>__</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>__</td>
<td>Yes</td>
</tr>
<tr>
<td>Westerlind et al&lt;sup&gt;33&lt;/sup&gt;</td>
<td>&lt;1hours/week</td>
<td>25-40</td>
<td>18-35</td>
<td>Self-reported</td>
<td>None x6 months</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>__</td>
</tr>
<tr>
<td>Schmitz et al&lt;sup&gt;38&lt;/sup&gt;</td>
<td>&lt;2x/week x6month</td>
<td>18-25</td>
<td>18-40</td>
<td>25-32 days</td>
<td>None x1 year</td>
<td>None x1 month</td>
<td>None</td>
<td>__</td>
<td>None</td>
<td>Yes</td>
<td>&lt;7 alcoholic beverages/week, not pregnant, nulliparous, no GYN disorders</td>
</tr>
<tr>
<td>Bentz et al&lt;sup&gt;34&lt;/sup&gt;</td>
<td>__</td>
<td>__</td>
<td>18-30</td>
<td>26-32 days</td>
<td>None x3 months</td>
<td>None x6 months</td>
<td>None</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>__</td>
</tr>
<tr>
<td>Campbell et al 2005&lt;sup&gt;2&lt;/sup&gt;</td>
<td>__</td>
<td>20-42</td>
<td>18-24</td>
<td>24-36 days, 10/12months</td>
<td>None x6 months</td>
<td>None x12 months</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>__</td>
</tr>
</tbody>
</table>

*Items left blank were not addressed by the study. Medical Conditions included diabetes, thyroid disorders, liver or kidney disorders, or uncontrolled hypertension.*
Table 3: Limitations of Selected studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Baseline characteristics</th>
<th>Type of Study</th>
<th>Intervention</th>
<th>Menstrual Cycle</th>
<th>Metabolite Levels</th>
<th>Additional Diet Changes</th>
<th>Sample size &amp; Dropout Rate</th>
<th>Control Group</th>
<th>Fitness Measurements</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al 2007</td>
<td>Ratio: Not measured PA: &lt;20min/day, 3x/wk, VO2max &lt;40 Body comp: BMI and WHR</td>
<td>RCT</td>
<td>Length: 12 weeks Supervised: Yes Prospective: Yes</td>
<td>Luteal phase, verified with saliva hormone levels</td>
<td>Not stratified to creatinine concentration</td>
<td>No diet changes.</td>
<td>Intervention: 14/17 completed 80% Control: 15/15 completed</td>
<td>No similar intervention for control group</td>
<td>VO2max</td>
<td>Caucasian females only</td>
</tr>
<tr>
<td>Pasagian-Macaulay et al 18</td>
<td>Ratio: Measured PA: No limitations Body comp: BMI only</td>
<td>RCT</td>
<td>Length: 20 weeks Supervised: Self-reported Prospective: Yes</td>
<td>Not standardized</td>
<td>Not stratified to creatinine concentration</td>
<td>Additional dietary intervention</td>
<td>Intervention: 84</td>
<td>Control: 90 Dropout rate not discussed</td>
<td>No similar intervention for control group</td>
<td>Measured by weight and BMI change</td>
</tr>
<tr>
<td>Westerlind et al 33</td>
<td>Ratio: Not measured PA: &lt;1hr/wk Body comp: Measured with hydrostatic weighing</td>
<td>Cross-over</td>
<td>Length: 16 weeks Supervised: Yes Prospective: Yes</td>
<td>Folicular and luteal phases, verified with daily urine hormone levels</td>
<td>Stratified to creatinine concentration</td>
<td>Additional dietary intervention</td>
<td>24/31 completed study</td>
<td>No control group</td>
<td>VO2max</td>
<td>No criteria set</td>
</tr>
<tr>
<td>Schmitz et al 38</td>
<td>Ratio: Not measured PA: &lt;2hrs/wk, fitness assessed by HR Body comp: Measured x-ray absorptiometry</td>
<td>Cross-over</td>
<td>Length: 15 weeks Supervised: Partially Prospective: Yes</td>
<td>Self-reported follicular phase</td>
<td>Stratified to creatinine concentration</td>
<td>No diet changes</td>
<td>15/28 completed study</td>
<td>No control group</td>
<td>Not measured, but also recorded lipid peroxidation markers</td>
<td>No criteria set</td>
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<tr>
<td>Bentz et al 34</td>
<td>Ratio: Not measured PA: No limitations Body comp: BMI only</td>
<td>Cross-over</td>
<td>Length: 2 weeks Supervised: Self-reported Prospective: Yes</td>
<td>Self-reported luteal phase</td>
<td>Stratified to creatinine concentration</td>
<td>Self-reported dietary logs recorded</td>
<td>77 women all completed study</td>
<td>No control group</td>
<td>Not measured</td>
<td>African American women excluded</td>
</tr>
<tr>
<td>Campbell et al 8</td>
<td>Ratio: Not measured PA: Either no PA or &gt;3-5 days/wk, VO2max either &gt;48 or &lt;40 Body comp: Sum of skin folds only</td>
<td>Cohort</td>
<td>Length: 12 months Supervised: Self-reported Prospective: Retrospective</td>
<td>Luteal and follicular phases, verified with salivary hormone levels</td>
<td>Not stratified to creatinine concentration</td>
<td>Self-reported dietary logs recorded</td>
<td>High fitness group: 17/18 completed Average fitness group: 13/18 completed</td>
<td>No control group</td>
<td>Not measured</td>
<td>Caucasian only</td>
</tr>
<tr>
<td>Validity Measure</td>
<td>Study Score</td>
<td>Study Score</td>
<td>Study Score</td>
<td>Study Score</td>
<td>Study Score</td>
<td>Study Score</td>
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<tr>
<td>---------------------------------------------------------------------------------</td>
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<tr>
<td>_</td>
<td>Campbell et al 2007(^{30})</td>
<td>Pasagian-Macaulay et al(^{18})</td>
<td>Westerlind et al(^{33})</td>
<td>Schmitz et al(^{38})</td>
<td>Bentz et al(^{34})</td>
<td>Campbell et al 2005(^{2})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were the inclusion and exclusion criteria well specified?</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was there a comparison/control group?</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If so, were groups similar at start of trial?</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Were the two groups randomly assigned by a valid method of randomization?</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Was exercise the only intervention?</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was there a similar intervention for the control group?</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Were the participants’ workouts supervised as opposed to self-reported?</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were the sample sizes greater than 30?</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were all patients who entered the trial properly accounted for and attributed at its conclusion? Were these patients included in the final calculations?</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were the results measured in change in 2/16 ratio?</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are the likely benefits worth the potential harms and costs?</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>TOTAL SCORE</strong></td>
<td><strong>8</strong></td>
<td><strong>8</strong></td>
<td><strong>6</strong></td>
<td><strong>6</strong></td>
<td><strong>5</strong></td>
<td><strong>4</strong></td>
<td></td>
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</tbody>
</table>