The Effects of Aerobic Exercise on Premenopausal Levels of Endogenous Sex Hormones and Urinary Estrogen Metabolites

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Dedication

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Abstract

The purpose of this doctoral project was to investigate the effects of aerobic exercise on breast cancer risk biomarkers such as endogenous sex hormone levels and urinary estrogen metabolites in premenopausal women. In this study, 391 healthy, sedentary, young eumenorrheic women were randomized to either an exercise intervention of 30 minutes of moderate-to-vigorous aerobic exercise five times a week for approximately 16 weeks (n = 212) or a usual-lifestyle sedentary control group (n = 179). Outcome measures were taken at baseline and follow-up.

The study described in Chapter 2 evaluated changes in serum levels of estradiol, estrone sulfate, testosterone, progesterone, and sex hormone-binding globulin (SHBG) via radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods. As expected, the intervention resulted in significant increases in aerobic fitness, lean body mass, and decreases in percent body fat in women in the exercise group. No significant changes in body weight were observed between or within groups. The major finding of this study was a significant decrease in progesterone in the exercise group, albeit this change was similar to that of the control group. The lack of significant changes in sex hormone or SHBG levels due to the intervention (without a concomitant weight change) between or within groups suggest this may not be a plausible mechanism by which physical activity decreases breast cancer risk in premenopausal women.

The second study described in Chapter 3 measured changes in urinary levels of estrogens (estrone [E₁], and estradiol [E₂]), and ten estrogen metabolites via liquid chromatography/tandem mass spectrometry (LC-MS/MS). In addition, the ratios of 2-
hydroxyestrone to 16α-hydroxyestrone (2-OHE\textsubscript{1}/16α-OHE\textsubscript{1}) and 2-hydroxyestrone to 4-hydroxyestrone (2-OHE\textsubscript{1}/4-OHE\textsubscript{1}) were calculated. Although no significant changes in urinary estrogens or their metabolites were found between groups, the 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} ratio in exercisers increased significantly ($P = 0.043$) compared to the control group ($P = 0.045$), even after adjustment for baseline values.

Collectively, the results of this doctoral project suggest the positive effects aerobic exercise allegedly has on premenopausal breast cancer risk may be mediated through hormonal mechanisms that involve changes in estrogen metabolism but not necessarily changes in endogenous sex hormone levels.
CHAPTER 1: LITERATURE REVIEW

I. Breast Cancer
   A. Relevance
   B. Risk Factors

II. Breast Cancer Risk
   A. Biomarkers
   B. Endogenous Sex Hormones and SHBG
      1. Experimental Evidence
      2. Observational Evidence
         a. Estradiol
         b. Estrone
         c. Progesterone
         d. Testosterone
         e. SHBG
III. Subjects and Methods................................................................. 85
IV. Results................................................................. 91
V. Discussion................................................................. 94

CHAPTER 4: DISSERTATION SUMMARY................................. 103

BIBLIOGRAPHY................................................................. 108

APPENDICES................................................................. 136
Appendix A-1 Radioimmunoassay Methods................................. 137
Appendix A-2 ELISA Method.................................................. 146
Appendix A-3 LC-MS/MS Method for Estrogen Metabolite Excretion.... 149
Appendix B  WISER Study Handbook................................. 153
Appendix C-1 SAS Code: Endogenous Sex Hormones and SHBG........... 200
Appendix C-2 SAS Code: Urinary Estrogen Metabolites...................... 227
List of Tables

CHAPTER 2: ENDOGENOUS SEX HORMONES AND SHBG

Table 2.1 Baseline Characteristics of Randomized Participants by Treatment Group 73
Table 2.2 Changes in Fitness, Body Weight, Body Composition, and Energy Intake 75
Table 2.3 Baseline, Follow-up, and Changes from Baseline in Sex Hormone and SHBG Levels 77

CHAPTER 3: URINARY ESTROGEN METABOLITES

Table 3.1 Baseline Characteristics of Randomized WISER Participants 98
Table 3.2 Effects of 16 weeks of Aerobic Exercise on Estrogen Metabolism of WISER Participants 99
List of Figures

CHAPTER 1: LITERATURE REVIEW

Figure 1.1 Steroidogenic Pathway in the Ovary……………………….. 56

CHAPTER 2: ENDOGENOUS SEX HORMONES AND SHBG

Figure 2.1 CONSORT Diagram Showing Participant Recruitment, Screening, Randomization, and Retention…………………. 79

CHAPTER 3: URINARY ESTROGEN METABOLITES

Figure 3.1 CONSORT Diagram Showing Participant Recruitment, Screening, Randomization, and Retention……………………….. 101

Figure 3.2 Changes in Baseline vs. Baseline in 2-OHE₁/16α-OHE₁ Ratio………………………………………………………………… 102
List of Appendices

A. METHODS AND ASSAYS

Appendix A-1 Radioimmunoassay Methods ........................................ 137
Appendix A-2 ELISA Method .......................................................... 146
Appendix A-3 LC-MS/MS Method for Estrogen Metabolite Excretion.  149

B. WISER STUDY HANDBOOK

Appendix B Table of Contents ...................................................... 155
Overview ..................................................................................... 157
General Data Collection Information ..................................... 165
Data Collection for Participants .............................................. 167
WISER Contact Information ..................................................... 182
Data Collection Forms ............................................................. 190

C. STATISTICAL ANALYSES

Appendix C-1 SAS Code: Endogenous Sex Hormones and SHBG……  200
Appendix C-2 SAS Code: Urinary Estrogen Metabolites ................. 227
CHAPTER 1:

LITERATURE REVIEW
I. BREAST CANCER

A. Relevance

Breast cancer continues to be the most widely diagnosed disease and second leading cause of death in women in the United States. In 2014, breast cancer is expected to account for 29% of all newly diagnosed cancer cases in women and 15% female mortality, which translates into 232,670 new cases of breast cancer and 40,000 deaths [1]. Despite significant improvements in the last three decades in 5-year survival rates (90% survival rate in 2003-09 vs. 75% survival rate in 1975-77), the incidence of breast cancer has remained stable since 2003 [1]. These statistics suggest great advances have been made in the diagnosis and treatment of breast cancer but much improvement is still needed in the prevention of the disease.

B. Risk Factors

As in the case with most cancers, age is one of the best predictors of breast cancer risk. The probability a woman will develop breast cancer within her lifetime is 1 in 8 or 12.3%, and the risk increases rapidly with each passing decade of life after 50 [1]. Specifically, the probability of being diagnosed with breast cancer at age younger than 50 is 1 in 53 and the odds increase to 21%, 84%, and 350% at age 50, 60, and 70 years old or older, respectively [1]. Other well-established risk factors include specific hereditary, genetic, environmental, reproductive, hormonal, and lifestyle factors.

For instance, positive family history of breast cancer, number and type of relatives (1st and 2nd degree) with breast cancer, and age at which these relatives became affected are hereditary risk factors associated with 5-10% of the total breast cancer burden [2]. Of
the breast cancer cases considered to be hereditary, at least 30% have been attributed to mutations in two major breast cancer susceptibility genes known as *BRCA1* [3] and *BRCA2* [4] genes. Although the prevalence of *BRCA1* and *BRCA2* gene mutations in the general population is 1 in 1000, accounting for less than 5% of all breast cancer cases, women with these mutations are 60-85% more likely to develop the disease [5, 6]. The likelihood of carrying a mutation in these susceptibility genes has been linked to early-onset breast cancer, breast and ovarian cancer, and Ashkenazi Jewish ancestry [7, 8].

Rare germline mutations in other high penetrance genes (relative risk > 5) such as *TP53*, *PTEN*, *SKT-11*, *MMR* genes, and *CDH1* have been recently associated with breast cancer syndromes such as Li-Fraumeni syndrome, Cowden/PTEN hamartoma tumor syndrome, Peutz-Jeghers syndrome, Lynch syndrome, and hereditary diffuse gastric cancer, respectively [9]. Although these syndromes are associated with less than 1% of all breast cancer cases, breast cancer is the most common malignancy among females carrying these mutations [9].

Another well-established but uncommon risk factor for breast cancer in women involves radiation exposure to the chest. Data from a newly published study suggests this risk is much larger than originally thought. Specifically, the study found women who had received radiation to the chest as children to treat cancers such as Hodgkin lymphoma and non-Hodgkin lymphoma, were 20 times more likely to develop breast cancer than average women [10]. In addition, 30% of women in the study developed breast cancer by the time they were 50 years old, and approximately 20% of those affected died of the
disease within ten years of being diagnosed [10]. Because of this deleterious side effect, high-dose radiation is no longer used to treat childhood Hodgkin lymphoma.

Reproductive characteristics such as early age at menarche (< 12 years old), older age at menopause (≥ 55 years old), nulliparity, and older age at first live birth (> 30 years old) have been shown to put women at higher risk for breast cancer [11]. Similarly, women with benign breast disease such as atypical hyperplasia [12] and history of one or more breast biopsies are considered to be at higher risk for breast cancer [11, 13, 14].

The use of exogenous sex hormones has long been associated with an increased risk for breast cancer. This is particularly the case of postmenopausal women who have used estrogen-plus-progestin hormone replacement therapy (HRT) [15]. The Women’s Health Initiative, a very large randomized clinical trial (n = 16,608) reported postmenopausal women who had used a combined estrogen-progestin HRT had a significantly higher hazard ratio (HR) for total (HR = 1.24, \( P < 0.001 \)) and invasive breast cancer (HR = 1.24, \( P = 0.003 \)) than women who did not use any kind of HRT [16].

In premenopausal women, the use of exogenous sex hormones in the form of combined estrogen and progestin oral contraceptives (OC) has not been regarded as an established risk factor. Results of case-control and cohort studies have either reported borderline effects or no association at all with breast cancer. For instance, a recent meta-analysis of 66 case-control and cohort studies (totaling 35,527 breast cancer cases and 180,318 controls) found premenopausal women who had ever taken OC had a slightly higher odds ratio (OR) (OR = 1.10, 95% confidence interval (CI): 1.03-1.18) than those who had never taken OC [17]. In addition, women who took OC the longest (> 10 years
vs. < 5 years) were at 17% greater risk, although non-significantly. In the Nurses’ Health Study (NHS), the largest prospective cohort to investigate the association between OC and female mortality, the use of OC by premenopausal women was found to be not significantly associated with a higher risk for breast cancer after 24 [18] or even 36 years of follow-up [19].

Lifestyle behaviors associated with breast cancer risk that are particularly amenable for intervention include alcohol consumption, physical inactivity, excess body weight, and breastfeeding.

The consumption of alcohol in habitual to moderate quantities, that is, up to one drink per day in women, has been associated with lower rates of mortality, coronary artery disease, diabetes mellitus, and stroke [20]. However, even in these low quantities, alcohol consumption has also been associated with a significantly higher risk for breast cancer. Specifically, a meta-analysis of 65 studies reported that women, regardless of whether they had ever smoked or not, increased their relative risk (RR) for breast cancer by 7.1% with each additional 10g per day of alcohol consumed (95% CI: 5.5-8.7%; \( P < 0.0001 \)) [21].

With a global prevalence of 39%, physical inactivity is estimated to be responsible for 10% of the incidence of breast cancer worldwide [22]. This is extremely relevant given that in a review of 73 studies conducted worldwide, women who were the most physically active compared to those who were inactive had a significant average risk reduction of 25% of breast cancer [23].
Similarly, excess body weight (mostly due to physical inactivity) has been strongly associated with breast cancer in women. Observational studies have revealed this association is mediated by menopausal status. For instance, in postmenopausal women, most large case-control studies (but only some cohort studies) have found a clear positive association between body mass index (BMI) and breast cancer risk, while in premenopausal women, most cohort studies have found the association to be inverse [24, 25].

Consistent with these observations, a meta-analysis of seven prospective studies totaling 337,819 women and 4,385 incident cases of invasive breast cancer, found nonlinear positive and inverse associations with breast cancer in post- and premenopausal women, respectively [26]. Specifically, postmenopausal women with a BMI greater than 31 had a 29% higher risk for breast cancer (95% CI: 1.03-1.60, \( P_{trend} = 0.001 \)) than women with a BMI of less than 21. In postmenopausal women, the main source of estrogen production comes from the aromatization of androgens in adipose tissue and therefore, higher fat mass levels (associated with higher BMI levels) are associated with higher estrogen levels [27]. In addition, in postmenopausal women excess weight is associated with lower levels of SHBG [28], which also leads to higher circulating levels of biologically active estrogens [29]. In contrast, premenopausal women with a BMI greater than 31 had a 56% lower risk (95% CI: 0.34-0.85, \( P_{trend} = 0.007 \)) for breast cancer than women with a BMI of less than 21. It has been hypothesized that obesity in young women is protective against breast cancer by inducing more frequent anovulatory
menstrual cycles and luteal phase defects [30, 31], which ultimately result in lower levels of estradiol and progesterone [32].

Unlike the other modifiable lifestyle choices, breastfeeding has been consistently associated with numerous and long-lasting benefits for both babies and mothers, especially as breastfeeding duration increases. Point-in-case, a meta-analysis of 32 studies ($n = 134,767$) found women who had ever breastfed were 14% less likely (95% CI: 0.84-0.89) to be at risk for breast cancer than women who never breastfed, even after the adjustment for parity [17]. Furthermore, the protective effect of breastfeeding on breast cancer risk increased to 28% (95% CI: 0.58-0.89) when mothers breastfed ≥ 12 months relative to non-breastfeeding women [17].

Finally, factors not yet considered to be established risk factors for breast cancer but that are currently being investigated include mammographic density, bone density, fracture risk, and endogenous sex hormone levels. In 1976, John N. Wolfe proposed a system of four categories based on mammographic density in which higher density correlated with higher risk [33, 34]. In fact, a recent study involving 2,392,998 women found a RR of 4.09 (95% CI: 3.6-4.6) for women in the extremely dense category [35]. Currently, the use of mammographic density to assess breast cancer risk has been limited and controversial due to possible “masking” of breast cancer by dense breast tissue [15]. Masking occurs when tumors in dense breast tissue are missed at a woman’s first mammographic examination but are eventually detected at subsequent follow-up examinations, creating the impression of a higher risk associated with mammographic density.
Bone density and fracture risk are also considered potential risk factors since they are believed to be surrogate markers for increased estrogen exposure. Higher estrogen exposure, in turn, is thought to be associated with increased risk for breast cancer. In general, studies in women have shown a positive and inverse association between breast cancer risk and bone density and bone fractures, respectively [15].

Evidence in support of using endogenous sex hormone levels to predict breast cancer risk continues to accumulate, especially for postmenopausal women. In this population, a positive association between breast cancer risk and endogenous sex hormone levels has been identified. For instance, in a current re-analysis of 18 prospective studies, the OR for postmenopausal women in the highest quintile for estradiol, estrone, and testosterone, compared to women in the lowest quintile, was 2.15 (95% CI: 1.87-2.46), 1.81 (95% CI: 1.56-2.10), and 2.04 (95% CI: 1.76-2.37), respectively [36]. Evidence of an association between endogenous sex hormones and breast cancer risk in premenopausal women will be reviewed in the following section.

II. BREAST CANCER RISK

A. Biomarkers

Due to the long latency of cancer, there are currently no prevention studies in which breast cancer is the measured outcome. The undertaking of this type of study would not only require an extraordinarily large study sample and long follow up time, but it would also be extremely costly. Alternatively, current research uses biomarkers for breast cancer risk as surrogate outcomes rather than the disease itself.
Biomarkers are biological factors thought to be involved in the casual pathway between exposure and cancer development. To date, the most well-studied biomarkers in breast cancer prevention research are endogenous sex hormones such as estrogens (estradiol, estrone, estrogen metabolites), progesterone, testosterone, and sex hormone-binding globulin (SHBG).

Other non-hormonal biomarkers of interest include insulin and insulin resistance, levels of metabolic hormones (i.e., leptin, adipokines), inflammatory markers (i.e., prostaglandins, C-reactive protein), immune function markers (i.e., natural killer cells, leukocytes, T helper cells), and oxidative stress markers (i.e., F2-isoprostanes, reactive oxygen species) [37].

B. Endogenous Sex Hormones and SHBG

In women of reproductive age, the ovaries are the main source of sex hormones. Steroidogenesis in peripheral tissues such as subcutaneous fat and skin, and physiologic and pathologic target sites such as the hypothalamus and cells of the breast and endometriosis are also important, especially in postmenopausal women and anovulatory premenopausal women [38].

In general, the ovaries secrete three major classes of sex hormones, namely the $C_{18}$-steroids estrone ($E_1$) and estradiol ($E_2$), the $C_{21}$-steroids pregnenolone, progesterone, and 17α-hydroxyprogesterone, and the $C_{19}$-steroids dehydroepiandrosterone, androstenedione, and testosterone, [39]. The main precursor for all of these sex hormones is cholesterol. In the preovulatory follicle, cholesterol can be derived from dietary plasma
lipoprotein cholesterol and de novo synthesis, while in the corpus luteum, cholesterol is derived from intracellular cholesterol esters stores within lipid droplets [38].

The first and rate-limiting step in ovarian steroidogenesis involves the translocation of cholesterol into the mitochondrion of ovarian theca cells by the steroidogenic acute regulatory protein known as StAR [40]. Once inside the mitochondrion, cholesterol is converted into pregnenolone by the cholesterol side-chain cleavage enzyme or P450scc [40]. Pregnenolone is then converted to progesterone, which in turn is converted to androstenedione by the action of enzymes 3β–hydroxysteroid dehydrogenase Δ^5,4 isomerase type II (3β-HSD-II) and 17α-hydroxylase/17,20-lyase (P450c17), respectively [41].

Androstenedione can then diffuse out of the theca cells into the granulosa cells where it is transformed to estrone by aromatase (P450arom) [42]. Alternatively, androstenedione diffuses into the circulation and reaches extraglandular tissues where it can mainly be converted into estrone by P450arom or (to a much lesser extent) into testosterone by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD-1) [27]. The conversion of androstenedione to estrone and the entry of cholesterol in mitochondrial thecal cells, are both critical steps in the formation of ovarian estradiol.

Finally, estrone is converted into biologically active estradiol by the action of 17β-HSD-1. It should be noted there are at least seven enzymes in the 17β-HSD family with overlapping activities, which are capable of converting E₁ into E₂ in both the ovary and peripheral tissues [43].
Once formed, estradiol and testosterone can enter the circulation in either their free (non-bound) form or bound to a protein like albumin or SHBG. SHBG is a plasma glycoprotein synthesized and secreted by the liver [44] that regulates the bioavailability of these two sex hormones by binding two molecules of steroid ligand at a time [29]. Figure 1.1 shows the steroidogenic pathway in the ovary.

I. Experimental Evidence

The role of estradiol and other sex hormones in the etiology of breast cancer has been substantiated by a plethora of studies in animals and human in vitro studies. For instance, in ovariectomized adult mice, administration of E$_2$ increased DNA synthesis of mammary epithelial cells [45]. Similarly, exposure of E$_2$ to human breast cancer cell lines Michigan Cancer Foundation-7 (MCF-7) [46] and ZR-75-1 [47] caused a marked increase in cell proliferation. Likewise, in the Noble rat model, administration of E$_1$ increased cell proliferation of mammary epithelium two-fold and perturbed cell kinetics leading to genetic instability [48].

Depending on the model used, the role of progesterone in breast carcinogenesis has been either supported or questioned. For example, in ovariectomized adult mice, the combined administration of E$_2$ and progesterone resulted in greater DNA synthesis than with either hormone alone [49] while in mouse mammary tumors, sole administration of progesterone was necessary to increase cell proliferation [50]. On the other hand, progesterone has been observed to decrease proliferation in primary cultures of normal human breast epithelium and breast cancer cells [51].
Studies on testosterone, similarly to progesterone, have provided evidence of both agonist and antagonist roles in mammary cell proliferation. For instance, in a study of mouse CH11 cell line, testosterone treatment significantly increased mammary epithelial growth, possibly through stimulation of the androgen receptor (AR) [52]. In contrast, in ovariectomized rhesus monkeys, the combined treatment of E\textsubscript{2} and testosterone resulted in approximately 40% reduction in E\textsubscript{2}-induced proliferation ($P < 0.002$) as well as abolishment of E\textsubscript{2}-induced augmentation of estrogen receptor alpha (ER-α) expression [53].

The opposing effects of testosterone can be explained by noting that at low estrogen levels (such as it is the case of postmenopausal women), testosterone and other androgens may have proliferative effects on mammary epithelial and cancer cells by direct binding to the ER-α and thus, mediate estrogenic functions. In contrast, at high estrogen levels (such as in the case of most premenopausal women), androgens may exert mainly antiestrogenic effects via binding to the AR resulting in the suppression of estrogen stimulation of breast epithelial and cancer cells [54].

Lastly, SHBG is thought to play a role in breast cancer etiology by regulating the amount of bioavailable (i.e., biologically active) fraction of estradiol and testosterone that can reach target cells [55]. More directly, SHBG has been reported to inhibit estradiol’s positive effect on cell proliferation [56, 57] while also completely reversing estradiol’s anti-apoptotic effect on MCF-7 cells [58].
2. Observational Evidence

Observational studies have shown a well-established positive association between endogenous levels of estrogens and testosterone and breast cancer risk in postmenopausal women [36]. This is not the case in premenopausal women.

To date, 14 prospective nested case-control studies have evaluated the association between breast cancer risk and premenopausal levels of estrogens, progesterone, testosterone, and SHBG. Most of these studies, especially those conducted in the 1980’s and 90’s, had limited statistical power as less than 100 cases of breast cancer were included. More recent updates to three of the four largest studies have greatly improved the ability to detect significant differences by extending the number of cases by approximately eight-fold.

a. Estradiol

To date, 12 nested case-control studies have investigated the association between premenopausal E$_2$ and breast cancer risk, making it the most well-studied sex hormone in the etiology of breast cancer. Of these studies, 11 have reported results based on levels of total E$_2$ [59-69], while four [60, 61, 64, 70] and two [59, 65] have additionally reported breast cancer risk based on free and bioavailable E$_2$, respectively. Most recently, updates in the number of breast cancer cases in three large cohort studies have allowed a more in-depth look at breast cancer risk based on cancer type (overall, invasive, and \textit{in situ}) and hormone receptor subtype (ER+, ER-, ER+/progesterone receptor (PR)+, and ER-/PR-) [60, 61, 64].
Despite the large amount of data now available, overall conclusions have been
difficult to formulate given the different approaches studies have used to account for
estrogen level fluctuations across the menstrual cycle. This variability is critical to
consider given E\textsubscript{2} levels have been shown to differ by more than hundred-fold over the
duration of the menstrual cycle [71]. Therefore, results from studies in which no
menstrual cycle matching or model adjustment was made, such as those of Bulbrook \textit{et al.} [70] and Kabuto \textit{et al.} [65], should be considered of low quality and unsuitable to
determine whether or not an association exists between estradiol levels and breast cancer
risk.

The remaining prospective studies have used different approaches to deal with
menstrual cycle variation including: matching to exact menstrual cycle day [67-69],
matching plus spline regression adjustment [59, 63, 66], and matching plus separate
analysis of follicular and luteal samples [60-62, 64].

Among the studies that only used exact menstrual cycle day matching, only one
reported significantly higher levels of estradiol in breast cancer women compared to
controls and a non-significant positive association with breast cancer [68], while the other
two observed the exact opposite associations [67, 69].

In contrast, all the studies that used a spline regression of estradiol levels on day of
cycle (in addition to matching), showed breast cancer cases had higher levels of estradiol
than controls, which in turn translated into a non-significant increase in breast cancer risk
[59, 63, 66].
Finally, in studies in which follicular and luteal estradiol samples were analyzed separately, the results have been inconsistent. For instance, two [60, 62] studies reported higher risk for all breast cancers with higher follicular levels of E\textsubscript{2} but two others found no association at all [61, 64]. Similarly, higher levels of luteal E\textsubscript{2} have been associated with lower risk for all types of breast cancer in three studies [61, 62, 67]; higher risk in one study [64], and no risk at all in another [60].

Conflicting results have also been reported for the risk of invasive, \textit{in situ}, ER+, and ER+/PR+ breast carcinomas and follicular and luteal levels of E\textsubscript{2}. For example, the risk of invasive breast cancer has been associated with higher follicular [60] and luteal [61] levels of E\textsubscript{2}, lower levels of luteal E\textsubscript{2} [60] or not at all [61]. Similarly, the risk for ER+/PR+ breast cancer has been associated with higher levels of follicular [60] and luteal [60, 61] E\textsubscript{2} or not all associated with follicular E\textsubscript{2} levels [61].

For \textit{in situ} breast carcinomas, Fortner \textit{et al.} found a positive association with follicular E\textsubscript{2} levels and an inverse association with luteal E\textsubscript{2} levels [61]. Finally, in the case of hormone subtype cancers, Kaaks \textit{et al.} reported the risk of ER+ breast cancers to increase with not only increasing levels of follicular E\textsubscript{2} but luteal E\textsubscript{2} levels as well [64].

The association between breast cancer risk and premenopausal levels of bioavailable and free estradiol has been investigated in two and four studies, respectively. As previously discussed, the results of Kabuto \textit{et al.} and Bulbrook \textit{et al.} provide questionable data due to the lack of control or adjustment for menstrual cycle variability in estradiol levels [65, 70]. In the remaining study, where matching and spline regression was used,
both follicular and luteal levels of bioavailable $E_2$ were found to be non-significantly associated with an increase for breast cancer [59].

In the case of free estradiol, three studies have yielded consistent results in terms of risk for developing ER+, ER+/PR+, and \textit{in situ} breast carcinomas. More specifically, increasing levels of both follicular and luteal free $E_2$ levels were associated not only with an increased risk for ER+ and ER+/PR+ breast cancer, but also with a decrease for \textit{in situ} breast carcinomas.

While the results of Fortner \textit{et al.} and Eliassen \textit{et al.} agree on a positive association between invasive breast cancer and luteal free $E_2$ levels, they differ in the direction of the association with follicular free $E_2$ levels [60, 61]. Similarly, opposite associations have been reported between follicular and luteal free $E_2$ levels and risk for all types of breast cancer [60, 61].

The disagreement in the results for overall and invasive breast cancer risk between the studies of Eliassen \textit{et al.} and Fortner \textit{et al.} is troublesome considering both used data from the Nurses’ Health Study II cohort (NHS-II), and the latter used an updated and considerably larger number of breast cancer cases than the former (514 cases vs. 197 cases).

In a 2011 meta-analysis of seven of the earliest prospective studies, the estimated breast cancer OR for a doubling of premenopausal $E_2$ was found to be non-significant regardless of whether follicular or luteal data was included in the analyses [72]. In 2013, another meta-analysis of seven prospective studies including two of the largest cohorts followed to date, namely the New York University Women’s Health study (NYU-WSH)
and NHS-II cohorts, found total and free E$_2$ levels to be significantly associated with premenopausal breast cancer risk [73]. Specifically, the relative risk for a doubling of total and free E$_2$ levels was 1.19 (95% CI: 1.06-1.35, $P_{\text{trend}} = 0.042$) and 1.17 (95% CI: 1.03-1.33, $P_{\text{trend}} = 0.014$), respectively.

It is important to keep in mind the more recent meta-analysis restricted data to only premenopausal cases in which breast cancer was diagnosed before age 50 and pooled together follicular and luteal E$_2$ measures to calculate the estimated odds ratio for breast cancer risk. While the updated reports of two of the other large prospective studies were not available at the time this meta-analysis was conducted, it is unlikely the inclusion of their data would have significantly changed the previously reported associations.

First of all, data from the updated Hormones and Diet in the Etiology of Breast Cancer (ORDET) study would have not been included in the meta-analysis considering the mean age at blood draw in the ORDET study was 43 years old, and the median follow-up time was 15.4 years, suggesting a postmenopausal and not premenopausal status at breast cancer diagnosis. Furthermore, when breast cancer cases in the updated European Prospective Investigation into Cancer and Nutrition (EPIC) study were restricted to those diagnosed before 50 years old, the positive associations between breast cancer risk and total and free E$_2$ levels were not found to be statistically significant any longer [64].

In conclusion, the results from 12 prospective, nested case-control studies and two meta-analyses do not provide significantly convincing evidence of a positive association between premenopausal total, free, or bioavailable E$_2$ levels and breast cancer risk.
\textit{b. Estrone}

Due to its less estrogenic activity, estrone has received far less consideration in the etiology of breast cancer compared to estradiol. In fact, only five [60-63, 69] of the 14 prospective, nested case-control studies investigating the association between premenopausal endogenous sex hormones and breast cancer risk have included E$_1$ in their analyses.

Results from two earlier studies nested both in the Campaign Against Cancer and Stroke cohort from Washington County, MD provided conflicting results. Specifically, while Wysowski \textit{et al.} showed women with breast cancer had lower levels of estrone than controls [69], Helzlsouer \textit{et al.} reported no significant differences in the levels of follicular and luteal estrone between cases and controls [62]. Both of these studies were very small, and with less than 25 cases, it is unlikely they were able to detect any statistical differences. In fact, Breslow and Day estimated a minimum of 250 case-control sets (1:3 matching ratio) would be necessary to detect a significant difference at 0.05 level and 80\% power [74].

In the EPIC study of 801 cases and 1,132 controls, a non-significant increase in risk was associated with higher levels of E$_1$ [63]. Similarly, in the NHS-II study, follicular E$_1$ levels were associated with a non-significant increase in breast cancer risk, but luteal levels were found to have the opposite association [60]. When the number of cases in the NHS-II study was updated from 197 to 514 in 2013, information on breast cancer overall and by hormone receptor subtype further elucidated the differences in risk based on menstrual cycle phase estrone levels [61].
In this updated study, Fortner et al. reported a non-significantly higher risk for ER+/PR+ and in situ breast cancer with higher levels of follicular E$_1$ and lower levels of luteal E$_1$. The risk for invasive breast cancer, on the other hand, was observed to decrease with both higher levels of follicular and luteal E$_1$. Similarly, a recent meta-analysis using all but the study by Wysowski et al. showed higher levels of follicular E$_1$ in premenopausal women to be associated with a significant increase in breast cancer risk overall [73]. Specifically, a doubling of follicular E$_1$ levels in premenopausal women translated into 27% higher risk for breast cancer (OR = 1.27, 95% CI: 1.05-1.54, $P_{\text{trend}} = 0.014$). Unfortunately, this meta-analysis did not include data for luteal E$_1$ levels, so the pooled estimate effect of follicular E$_1$ levels on breast cancer risk is currently unavailable.

c. Progesterone

Results from observational studies have not provided clear evidence of an association between premenopausal luteal progesterone levels and breast cancer risk. For instance, in four nested case-control studies, women with breast cancer had non-significantly higher levels of luteal progesterone than controls, which translated into a non-significant increase in risk for breast cancer including ER+/PR+ subtype [60, 62, 64, 67].

In contrast, in seven other nested case-control studies [60, 61, 63, 64, 68, 69, 75], an inverse relationship with breast cancer risk was found, although the association was only significant in the ORDET study of Micheli et al. (OR = 0.12, 95% CI: 0.03-0.52, $P_{\text{trend}} = 0.005$) [75]. However, when Micheli et al. restricted their analysis to women with menses without marked irregularities, the inverse association lost statistical significance ($P_{\text{trend}} = 0.077$). Furthermore, when the number of breast cancer cases in the ORDET cohort was
updated in 2013, the association not only ceased to be significant but also reversed
direction (OR = 1.16, 95% CI: 0.6-2.3, \( P_{\text{trend}} = 0.75 \)) [67].

When the NHS-II and EPIC cohorts were updated in 2013 and 2014, respectively, no
change in direction of the association previously reported was evident. The magnitude of
the association, however, was attenuated but the inverse association remained non-
significant. Finally, a meta-analysis by the Endogenous Hormones and Breast Cancer
Collaborative Group in 2013 concluded premenopausal, luteal progesterone levels were
not associated at all with the risk for breast cancer (OR = 1.00 95% CI: 0.92-1.09, \( P_{\text{trend}} =
0.93 \)) [73].

d. Testosterone

While the evidence implicating premenopausal progesterone levels in the etiology of
breast cancer has been weak and inconclusive, data on testosterone has consistently
shown a positive and statistically significant association. In fact, of the ten prospective,
nested case-control studies conducted to date [59-61, 63, 64, 67-69, 75, 76], only two [60,
69] have failed to show a difference in risk between cases and controls based on the
levels of total, free, and bioavailable testosterone.

The majority of the studies showing women with breast cancer with higher levels of
total [59, 63, 64, 76], free [64, 67, 76], and bioavailable testosterone [59] found the
associations to be statistically significant. Specifically, the relative risk of breast cancer
reported with highest vs. lowest levels of total testosterone in these studies has ranged
from OR = 1.73 (95% CI: 1.2-2.6; \( P_{\text{trend}} = 0.01 \)) to 3.3 (95% CI: 1.5-7.5; \( P_{\text{trend}} = 0.006 \)).
The relative risk reported by studies for women in the highest vs. lowest levels of free
testosterone has ranged from OR = 1.13 (95% CI: 1.01-1.26, \( P_{\text{trend}} = 0.04 \)) to 2.4 (95% CI: 1.1 to 5.1; \( P_{\text{trend}} = 0.03 \)). Finally, the relative risk for women in the highest vs. lowest quartiles of bioavailable testosterone levels reported by Dorgan et al. was OR = 4.2 (95% CI: 1.6-10.9, \( P_{\text{trend}} = 0.002 \)) [59].

In terms of invasive, in situ, ER+, and ER+/PR+ breast cancer, only three studies have investigated the association with testosterone levels. In all of these studies, higher levels of total and free testosterone were associated with higher risk for invasive [61, 76], in situ [61], ER+ [64, 76], ER- [64], ER+/PR+ [64], and ER-/PR- [64] breast carcinomas. However, only Zeleniuch-Jacquotte et al. were able to report a statistically significant association for total and free testosterone with invasive (OR = 1.5, 95% CI: 1.1-2.2, \( P_{\text{trend}} = 0.03 \) and OR = 1.2, 95% CI: 1.0-1.4, \( P_{\text{trend}} = 0.02 \), respectively) and ER+ (OR = 2.4, 95% CI: 1.2-4.6, \( P_{\text{trend}} = 0.01 \) and OR = 1.6, 95% CI: 1.2-2.2, \( P_{\text{trend}} = 0.003 \), respectively) cancers.

With the exception of the most updated data from the EPIC and ORDET cohorts, a recent meta-analysis found a doubling of total and free testosterone levels to be associated with an OR= 1.18 (95% CI: 1.03-1.35, \( P_{\text{trend}} = 0.018 \)) and OR= 1.14 (95% CI: 1.01-1.28, \( P_{\text{trend}} = 0.031 \)), respectively [73]. In the EPIC study, the inclusion of new breast cancer cases resulted in the attenuation of the positive association between breast cancer risk and total and free testosterone levels (OR = 1.14, 95% CI: 1.02-1.28, \( P_{\text{trend}} = 0.02 \) and OR = 1.13, 95% CI: 1.01-1.26, \( P_{\text{trend}} = 0.04 \), respectively) although it remained significant [64]. In the case of the ORDET study, the previously non-significant association reported between breast cancer risk and total testosterone remained non-
significant, while that of free testosterone reached statistical significance (OR = 2.4, 95% CI: 1.5-5.1, $P_{trend} = 0.03$ [67].

Overall, data from prospective studies and results from one meta-analysis clearly support the positive, significant association between breast cancer risk and premenopausal total and free testosterone levels.

**e. SHBG**

The effect of SHBG on premenopausal breast cancer risk has been evaluated in six small [59, 62, 65, 68, 70, 75] and six large [60, 61, 63, 64, 67, 76] nested case-control studies. In three of these studies, women with breast cancer had similar levels of SHBG than controls and therefore, no association with breast cancer risk was found [62, 64, 65]. In four other studies, breast cancer cases had higher levels of SHBG than controls as well as a non-significant risk increase for invasive, ER-, ER+/PR+, and ER-/PR- breast cancers [60, 61, 64, 68]. In contrast, three small [59, 70, 75] and five large [61, 63, 64, 67, 76] studies reported women with breast cancer had non-significantly lower levels of SHBG than controls which correlated with a non-significant risk reduction of 5-52% for overall breast cancer, 7% for ER+ breast cancer, and 20% for *in situ* breast cancer.

Finally, in a recent meta-analysis [73] of eight prospective studies, including 767 cases of breast cancer and 1699 controls, premenopausal SHBG levels were not associated with breast cancer risk (OR= 1.07, 95% CI: 0.94-1.23). Since the time this meta-analysis was published, updated data from the ORDET [67] and EPIC [64] cohorts have become available, and their results support the evidence of an inverse association with breast cancer risk, albeit not statistically significant.
Overall, data from most prospective studies and this meta-analysis seem to suggest there is an inverse association between premenopausal levels of SHBG and breast cancer risk; however, to date, not one study has reported a statistical significant association.

C. **Estrogen Metabolites**

In the human body, native E$_2$ undergoes biotransformation to E$_1$ through oxidation at the C17 position. While this process is reversible, the formation of E$_1$ is largely favored as it occurs rapidly compared to the reduction of E$_1$ to E$_2$.

Phase I metabolism of estrogens involves irreversible oxidation at either the A-ring or D-ring. Hydroxylation of E$_1$ and E$_2$ at the A-ring in C2 position yields 2-hydroxyestone (2-OHE$_1$) and 2-hydroxyestradiol (2-OHE$_2$), respectively. Similarly, hydroxylation of E$_1$ and E$_2$ at the A-ring in C4 position yields 4-hydroxyestone (4-OHE$_1$) and 4-hydroxyestradiol (4-OHE$_2$), respectively. Alternatively, oxidation at the D-ring in C16α position yields 16α-hydroxyestrone (16α-OHE$_1$) and estriol (E$_3$), respectively. Figure 1.1 shows the steroidogenic pathway in the human ovary.

The tissue-specific hydroxylases responsible for these oxidations belong to the cytochrome P$_{450}$-dependent family. Specifically, CYP1A1, CYP1B1 (breast), and CYP1A2 (liver) catalyze the oxidation of E$_1$ into 2-OHE$_1$ and 2-OHE$_2$ while CYP1A1, CYP3A5 (breast), CYP3A4, and CYP3A7 facilitate the 16α-hydroxylation [77]. In contrast, a much smaller fraction of E$_1$ is hydroxylated at the 4C position by CYP1B1 (breast) and CYP1A2 (liver) enzymes [77].

Further oxidative metabolism of these hydroxyestrogens leads to the formation of 2- and 4-quinones that can cause DNA damage. In particular, 4-quinones form unstable
depurinating adducts with adenine and guanine. These adducts undergo spontaneous depurination causing the formation of apurinic sites, which in turn undergo error prone DNA repair with resultant point mutations [78]. In contrast, the 2-quinones form relatively stable DNA adducts and depurination rarely occurs [79]. Additionally, the 2- and 4-quinones can be reduced to semiquinones by cytochrome P<sub>450</sub> reductase resulting in a redox cycle that generates reactive oxygen species such as superoxide, which causes oxidative DNA damage [80].

Phase II metabolism involves the methylation of 2- and 4-hydroxyestrogens by the catechol-O-methyltransferase (COMT) enzyme into the 2-methoxyestrogens (2-methoxyestrone [2-MeOE<sub>1</sub>] and 2-methoxyestradiol [2-MeOE<sub>2</sub>]) and the 4-methoxyestrogens (4-methoxyestrone [4-MeOE<sub>1</sub>] and 4-methoxyestradiol [4-MeOE<sub>2</sub>]), respectively. Finally, parent estrogens (E<sub>2</sub> and E<sub>1</sub>) and hydroxyestrogens can undergo detoxification through glucuronidation, sulfonation, and/or O-methylation with the resulting products becoming water-soluble and therefore excretable in urine or feces [81, 82].

1. Experimental Evidence

The first suggestion that the manner in which estrogens are metabolized had an etiological significance in breast cancer was first introduced by Thomas Dao in 1979 [83]. *In vitro* studies using human breast cancer tissue, as well as animal studies in mice and rats, have provided supporting evidence that estrogen metabolites may in fact play a role in the initiation and progression of breast cancer.
For instance, the 2-hydroxyestrogens, due to their reduced binding affinity to the ER compared to E\textsubscript{2}, are capable of exhibiting both weak agonist and partial antagonistic roles [84, 85]. Specifically, in estrogen receptor-dependent MCF-7 and T47D cell lines, both 2-OHE\textsubscript{1} and 2-OHE\textsubscript{2} induce cell proliferation rate [86-88] while also suppressing the mitogenic effect of E\textsubscript{2} on these cells [86, 89, 90].

Conversely, 16α-OHE\textsubscript{1} binds tightly and covalently to the ER [91] and increases cell proliferation in MCF-7 [86, 89] and T47D cell lines [86]. In comparison, 4-OHE\textsubscript{2} exhibits a higher affinity for the ER than 2-OHE\textsubscript{2} (but less than E\textsubscript{2}) [84, 85, 88] and can induce tumor cell proliferation in MCF-7 cell line [88].

While both the 2-hydroxyestrogens (2-OHE) and 4-hydroxyestrogens (4-OHE) can undergo metabolic redox cycling and cause oxidative damage to both DNA and other cellular constituents, the 2-OHE have not shown the carcinogenic activity that has been reported for 4-OHE\textsubscript{2} [92]. This difference can be explained in part by the fact 2-OHE have a faster clearance \textit{in vivo} [93], are more readily inactivated by COMT [89, 90, 94], are less hormonally potent [84, 88-90], and dissociate away from ER more rapidly than the 4-OHE [95].

In contrast, the 2-methylestrogens (2-MeOE) and 4-methylestrogens (4-MeOE) bind weakly to the ER and exhibit < 3% of the affinity of E\textsubscript{2} [85]. Interestingly, in MCF-7 cell line, 2-MeOE\textsubscript{1} did not have a significant effect on tumor cell growth [90], while 2-MeOE\textsubscript{2} was a potent inhibitor of cell growth [87, 96] and angiogenesis [96]. Finally, in MCF-7 cells, E\textsubscript{3} promoted tumor cell proliferation with slightly less potency than E\textsubscript{2} [89], while in the T47D cell line, its potency was much more than E\textsubscript{2} [86].
2. Observational Evidence

The association between estrogen metabolism and breast cancer risk in postmenopausal women has been mixed. For instance, one case-cohort [97] and one nested case-control study found some significant associations [97, 98] while another nested case-control study found none [99]. Furthermore, a combined analysis [100] and a systematic review [101] found postmenopausal women with the highest 2-OHE$_1$/16α-OHE$_1$ ratio to have virtually the same risk as those with the lowest 2-OHE$_1$/16α-OHE$_1$ ratio.

In premenopausal women, five prospective studies have investigated the association between breast cancer risk and estrogen metabolism using blood [102] and urine samples [60, 103-105]. The earliest investigation, a case-cohort study of 38 cases and 597 controls conducted in the Island of Guernsey, found women with breast cancer had lower levels of urinary parent estrogens and E$_3$ than controls, which were associated with a non-significant 60% and 30% reduction in breast cancer risk, respectively [103]. Similarly, in the much larger case-control NHS-II study, women with breast cancer had significantly lower levels of urinary parent estrogens than controls while the 50% reduction in breast cancer risk was found to be non-significant [60].

In the case of 2-OHE$_1$, two nested case-control studies found a slight positive association between breast cancer risk and urinary [105] and serum [102] levels of 2-OHE$_1$, respectively. In contrast, the same two studies found higher urinary and serum levels of 16α-OHE$_1$ to be associated with a greater risk of breast cancer, albeit also non-significantly.
When looking at the ratio of 2-OHE$_1$ to 16α-OHE$_1$, all [60, 104, 105] but one [102] study, have associated a higher 2-OHE$_1$/16α-OHE$_1$ ratio with a lower risk for breast cancer. Specifically, a higher urinary 2-OHE$_1$/16α-OHE$_1$ ratio was non-significantly associated with a 10% [60], 25% [104], or even 45% [105] risk reduction in breast cancer. Of these estimates, the one by Eliassen et al. is likely to be the most accurate as it analyzed considerably more breast cancer cases ($n = 247$) than either of the Island of Guernsey studies ($n = 38$ and $n = 60$) and used a newer lab method based on high-performance liquid chromatography-tandem mass spectrometry assay (HPLC-MS/MS).

In contrast to enzyme immunoassays (EIA), HPLC-MS/MS has shown to be a more sensitive, specific, accurate, and reproducible assay method [106]. With the use of this new lab technology, Eliassen and colleagues were able to analyze not only the parent estrogens, 2-OHE$_1$ and 16α-OHE$_1$ but also ten other urinary metabolites including 2-OHE$_2$, 4-OHE$_1$, 2-MeOE$_2$, 2-MeOE$_1$, 4-MeOE$_2$, 4-MeOE$_1$, 2-Hydroxyestrone-3-methyl ether, 16-Ketoestriadiol, 16-Epiestriol, E$_3$, and 17-Epiestriol.

Results from this study show that in control women the most abundant parent estrogen is estrone (15% of total estrogen metabolites), while the most abundant estrogen metabolites are 2-OHE$_1$ (27%) and A/E E$_3$ (18%) [60]. None of these metabolites were individually associated with a significant decrease or increase in breast cancer risk. However, a significant positive association was found between breast cancer risk and the ratio of 16-pathway metabolites to parent estrogens (top vs. bottom quartile RR = 1.61; 95% CI: 0.99-2.62). None of the other ratios that were calculated such as 2-
pathway/parent estrogens, 4-pathway/parent estrogens, and 2-OHE$_1$/16α-OHE$_1$ showed a statistical significant association with breast cancer risk [60].

As has been the case with retrospective case-control studies, most prospective studies have relied on the 2-OHE$_1$/16α-OHE$_1$ ratio to assess the effect of estrogen metabolism on breast cancer risk. The fact most studies have restricted their analysis to only these two estrogen metabolites has been due in part to laboratory convenience as a new commercial EIA became available in the mid-1990s. At the time, this assay allowed separate measurement of 2-OHE$_1$ and 16α-OHE$_1$ and manual calculation of their ratio while avoiding the complicated and invasive measurement of previously used radiolabel tracers [107].

EIA methods have gradually been replaced by HPLC-MS/MS methods as studies have sought to improve the scope and accuracy of estrogen metabolism analyses. This, however, has not been translated into a more frequent detection of statistically significant associations. Aside from methodological advances, no statistical relevance has been found in any prospective studies regardless of study size and type (case-cohort vs. nested case-control), bio-specimen used (serum vs. urine), duration of urine collection (spot sample [60, 104], 12-hr sample [105], and 24-hr sample [103]), or type of statistical analyses employed (model adjustment for menstrual cycle phase [103], case-control matched on menstrual cycle phase [102, 104], and specific collection on luteal phase [60, 105]).
Overall, prospective studies in premenopausal women have failed to find statistically significant associations between estrogen metabolite levels and breast cancer risk and therefore, the association remains weakly suggestive.

**D. Genetic Variation in Estrogen Biosynthesis and Metabolism**

Polymorphisms in genes encoding for the enzymes involved in estrogen biosynthesis and metabolism, namely the cytochrome P450 oxygenases, the hydroxysteroid dehydrogenases, and COMT, have been associated with breast cancer risk. While polymorphisms in these genes may be associated with breast cancer risk by altering the expression levels of sex hormones and estrogen metabolites, evidence of this association for most of them has been inconclusive, largely due to small sample sizes and variability between populations [108].

Despite encoding for the mitochondrial enzyme responsible for the initial and rate-limiting step in ovarian steroidogenesis, genetic variation in the CYP11A1 gene has not been extensively studied. To date, most studies have concentrated on pentanucleotide [(TAAAA),n] repeat polymorphisms in Chinese women. The first study to assess breast cancer risk based on the number of TAAAA repeats in the CYP11A1 gene was the Shanghai Breast Cancer Study, a population-based case-control study of 1015 breast cancer cases and 1082 controls [109]. In this study, three common alleles with 4, 6, and 8 repeats were identified, accounting for nearly 99% of total alleles detected in the study population. Compared to women homozygous for the 4-repeat allele, women carrying the 8-repeat allele had an elevated risk of breast cancer (OR= 1.8, 95% CI: 1.2-2.7) and the risk was even greater for those who were homozygous for the risk allele (OR= 3.4, 95%
Furthermore, this study showed a positive association between CYP11A1 genotypes and breast cancer risk in both premenopausal and postmenopausal women. Since then, other studies have revealed breast cancer risk may be also be related to other variants located upstream of the coding region in Chinese women [110] and CYP11A1 haplotypes in a study of multiethnic populations [111]. Furthermore, in another study of Chinese women, those who were homozygous carriers of the 4-repeat allele relative to non-carriers were at a decrease risk for breast cancer (OR= 0.61, 95% CI: 0.39-0.96), while those homozygous carriers of the 6-repeat allele relative to non-carriers were at a significant increased risk (OR= 1.42, 95% CI: 1.03-1.97). [112].

Genetic variation in CYP17A1, the gene encoding for the enzyme converting progesterone to 17β-hydroxyprogesterone and subsequently to androstenedione, has been associated with breast cancer risk in some ethnic populations but not in others. For instance, increased breast cancer risk and single nucleotide polymorphisms (SNPs) in the CYP17A1 gene have been reported in studies of Thai [113], Canadian [114], German [115], Swedish [116], and American-Asian, African-American, and Latino women [117]. In contrast, there have been studies conducted in German [118], Swedish [119], Portuguese [120], Australian [121], and American [122] women that have not found evidence of an association with breast cancer risk.

Similarly, inconclusive results have been reported in studies investigating genetic variations in the gene CYP19A1 responsible for encoding for aromatase – the enzyme that catalyzes the conversion of androgens androstenedione and testosterone to estrone and estradiol, respectively. One SNP believed to interfere with RNA stability, namely the
rs10046 polymorphism, has been associated with increased breast cancer risk in three small studies [123-125], but not in other larger studies like the Shanghai Breast Cancer and the NHS-II study [126, 127]. Furthermore, no association was found in two meta-analyses involving Caucasian European (5,356 cases, 7,129 controls) and multiethnic populations (7,998 cases, 12,100 controls) [128, 129]. In contrast, the polymorphisms W39R, T201M, R264C, and M364T have been reported to be associated with decreased enzymatic activity and thus, increased breast cancer risk [130]. Specifically, the R39 allele has been associated with breast cancer risk in premenopausal Japanese women with late-age parity or high BMI [131], while increased risk was observed in Korean women who carried the variant C264 and consumed alcohol regularly [132]. Interestingly, a meta-analysis of 22 studies involving 10,592 cases and 11,720 in Asian and Caucasian women however, did not find a significant association between any of these SNPs and breast cancer risk [133]. Finally, numerous studies have investigated the association between an intronic tetranucleotide repeat (TTTA)_n in the CYP19A1 gene and breast cancer risk. Some studies have found a significant association in Caucasian, Japanese, Chinese, Brazilian, and Nigerian women [134-140] while other studies have not found a similar association in Asian and American women [141-143]. Two large meta-analyses however, did find an association between (TTTA)_{12} and decreased breast cancer risk and (TTTA)_{10} and increased risk [133, 144].

Other enzymes of importance in the biosynthesis of estrogen include the hydroxysteroid dehydrogenases 3β-HSD, which convert pregnenolone to progesterone, and 17β-HSD, which converts androstenedione to testosterone and estrone to estradiol.
Currently, no studies have substantiated a possible role for 3β-HSD polymorphisms in breast cancer susceptibility. In contrast, a particular polymorphism in the HSD17β gene has been associated with decreased risk for breast cancer. Specifically, a polymorphism (A to G, rs605059) in exon 6 leads to an amino acid substitution of Ser to Gly [145]. A recent meta-analysis of 9 studies involving 13,987 cases and 17,066 controls found no significant association between this SNP and breast cancer risk [146]. However, a subgroup analysis of this data revealed a significant inverse association in Caucasians for G/G vs. A/A (OR= 0.91, 95% CI: 0.83-1.00), G/A vs. A/A (OR= 0.92, 95% CI: 0.85-0.99), and G/G +G/A vs. A/A (OR= 0.92, 95% CI: 0.86-0.98). Although this meta-analysis did not find a publication bias or significant heterogeneity, it had some important limitations including small samples of Asian and African-American women and lack of stratification based on other risk factors (age, smoking status, etc.).

Similar to the CYP genes involved in estrogen biosynthesis, genetic variation in the CYP genes involved in estrogen metabolism such as CYP1A1, CYP1A2, CYP3A4, and CYP1B1 have been studied extensively in terms of their association with breast cancer risk.

As previously mentioned, CYP1A1 is a monooxygenase that catalyzes the conversion of estrogens predominantly to their 2-catechol estrogens in extrahepatic tissues. While several variant alleles have been identified, two of them (T3801C and A2455G) have been reported to increase the risk for breast cancer by significantly elevating enzymatic activity compared to the wild-type genotype [147, 148]. However, a recent meta-analysis of 23 studies involving 10,520 cases and 14,567 controls found no
significant associations between those heterozygous or homozygous for the T allele and breast cancer risk regardless of ethnicity and menopausal status [149]. Similarly, another meta-analysis reported no association between breast cancer risk and the variant T3801C or the polymorphisms T3205C and C2453A[150]. The only significant association found in this meta-analysis was for Caucasian women who were homozygous carriers for the A2455G G allele. Specifically, women of Caucasian origin carrying this SNP were found to be at significantly higher risk for developing breast cancer (pooled OR= 2.2, 95% CI: 1.3-3.8).

Mixed results have also been reported in studies of polymorphisms in the CYP1A2 and CYP3A4 genes, which are mainly expressed in the liver and favor 2-hydroxylation of estrogen. For example, in one multiethnic cohort study, women who were homozygote carriers of rs762551 A ➔ C in the CYP1A2 gene showed increased risk for breast cancer [151], while women carrying this SNP were not found to be at higher risk in the Shanghai Breast study [152]. Consistent with the null results found in the Shanghai Breast Study, a meta-analysis of nine studies comprising 7,580 cases and 10,020 controls found no association between this SNP and breast cancer risk [153]. Likewise, the Human Genome Epidemiology review and meta-analysis of 11 studies (3,810 cases and 3,173 controls) found the G allele and G carrier of the CYP3A4 polymorphism not to be significantly associated with breast cancer risk [154].

In contrast to the relative few polymorphisms found in other CYP genes, more than 300 polymorphisms have been identified for CYP1B1. In particular, the R48G, A119S, V432L, and N453 SNPs have been associated with higher 4-hydroxylation activity
compared to the wild-type CYP1B1 [155]. In case-control studies, these SNPs have been associated with increased breast cancer risk in Polish [156], French [157], Chinese [158], and American white and black women [159], while three meta-analyses did not found an association with breast cancer risk [160-162].

Finally, COMT has been extensively studied, as it is responsible for the inactivation of catechol estrogens through esterification and thus, the prevention of estrogen quinone-DNA adducts and reactive oxygen species. The polymorphism occurring at codon 158 of COMT results in the substitution of Val for Met, which seems to determine high- and low-activity alleles of the enzyme [163]. Specifically, in a *in vitro* study, the Met allele (GG vs. AA) coding for the low-activity and heat-labile enzyme was found to be four to five times less effective in methylating the catechol estrogens than the Val allele [164].

The numerous meta-analyses that have investigated the association between this SNP and breast cancer risk have reported conflicting results, mainly due to the ethnicities of the populations investigated. For example, in two meta-analyses restricted to Chinese women (4,626 cases, 5637 controls), significant risk for breast cancer was associated with the genetic models A/A vs. G/G (OR = 1.59, 95% CI: 1.12-2.27); A/A vs. G/A + G/G (OR = 1.62, 95% CI: 1.14-2.29); and A vs. G (OR = 1.15, 95% CI: 1.00-2.32) [165, 166]. Only one of these meta-analyses performed a subgroup analysis based on menopausal status and found the SNP presented a significant increased risk in the premenopausal group (A/A vs. G/A +G/G: OR = 1.94, 95% CI: 1.03-3.63) whereas in postmenopausal women, no significant increase in risk was associated with any of these genetic models [166]. In contrast, another meta-analysis including studies conducted in
Asian populations other than Chinese (i.e., Korean, Indian, Thai, and Asian American) found no association between this SNP and breast cancer risk [167].

Lastly, the most comprehensive meta-analysis to date, including 33 studies in Caucasians, 18 in Asians, and 5 in mixed ethnicities totaling 34,358 cases and 45429 controls, found no overall positive relationship between the COMT polymorphism and breast cancer risk [168]. Subgroup analyses revealed no significant associations regardless of ethnicity, menopausal status, or the source of controls.

Overall, further studies employing more homogenous populations and larger sample sizes are needed to substantiate the role of genetic variation in any of the genes involved in estrogen biosynthesis and metabolism and breast cancer pathogenesis.

III. PHYSICAL ACTIVITY AND BREAST CANCER RISK

The observation that physically active women, compared to sedentary or less active women, had a lower prevalence of breast cancer was first reported by Frisch and colleagues in 1985 in a study of young, female college athletes [169]. Since then more than 90 studies worldwide have studied the association between physical activity and breast cancer risk in women [23, 170, 171]. Overall, these studies provide convincing evidence of an inverse relationship between physical activity (PA) and breast cancer risk.

For instance, in a recent literature review, 33 cohort and 40 case-control studies found all physically active women (pre- and postmenopausal women combined), compared to the least active women, had a 30% and 20% average reduction in risk for breast cancer, respectively [23]. Of the 73 studies reviewed, 29 found a statistically significant risk
reduction [172-200]; eight found a borderline significant risk reduction [201-208]; 14 observed a non-significant risk reduction [209-222]; 19 reported null results [174, 223-240]; and only three found a non-significant increased risk for breast cancer among the most physically active women [241-243].

Among the types of physical activity reported, engagement in recreational and household activity resulted in the greatest breast cancer risk reduction (21% average), followed by transportation (walking/cycling) and occupational activities (18% and 13%, respectively).

In terms of intensity, moderate physical activity was associated with a risk reduction of 15% while vigorous physical activity was associated with a slightly greater risk reduction of 18%. Similarly, increasing duration of physical activity was found to offer the most risk protection. Specifically, 2-3 hours per week of moderate-to-vigorous physical activity was associated with a 9% risk reduction while 6.5 hours per week was associated with a 30% risk reduction.

On the effect of physical activity performed during different periods of life, breast cancer risk decreased by 16% during adolescence; 8% during early adulthood (20s); 15% during middle adulthood (30s/40s); and 17% during late adulthood (older than 50 years).

Finally, a smaller portion of these studies has investigated the effect modification of menopausal status. Specifically, 25 studies have analyzed data from premenopausal and postmenopausal women separately, while nine and 14 studies have exclusively focused on premenopausal and postmenopausal women, respectively. Data from these studies suggest a much stronger, inverse association between PA and breast cancer risk in
postmenopausal women than premenopausal women. In postmenopausal women, 87% of these studies found an inverse association between PA and breast cancer risk and 13% found no association at all. In contrast, 73% of the studies in premenopausal women the association between PA and breast cancer risk to be inverse; 24% found no association, and 3% of them the association to be positive albeit non-significantly.

Furthermore, a greater proportion of studies reporting an inverse association between PA and breast cancer risk have found the association to be statistically significant in postmenopausal women than in premenopausal women. For example, four of 16 cohort studies and 11 of 23 case-control studies found a significant risk reduction with increasing PA levels, while only two cohort and four case-control studies found the association to be statistically significant in premenopausal women.

A recent review in 2014 of 14 case-control and seven cohort studies further support these findings by reporting the only three studies that did not find a significant reduction in breast cancer risk with increasing PA were those in which the sample population was exclusively premenopausal [244]. However, when pre- and postmenopausal data that was analyzed separately was included, the overall odds ratio for case-control and cohort studies in premenopausal women was found to be statistically significant (OR = 0.84, 95% CI: 0.81-0.88 and OR = 0.61, 95% CI: 0.59-0.63, respectively).

Overall, there is enough evidence to suggest and support the observation that increasing levels of PA, especially of moderate-to-vigorous intensity, result in significant decreases in breast cancer risk in both premenopausal and postmenopausal women.
IV. PHYSICAL ACTIVITY AND HORMONAL RISK FACTORS

A. Endogenous Sex Hormones and SHBG

One of the proposed mechanisms by which physical activity is thought to mediate breast cancer risk is through changes in endogenous levels of sex hormones and SHBG. In the case of postmenopausal women, evidence from a re-analysis of 18 prospective studies has shown a significant association exists between higher serum levels of estradiol, estrone, and testosterone and postmenopausal breast cancer risk [36]. Evidence of a similar association in premenopausal women is scarce. To date, three cross-sectional [245-247], three prospective [248-250], and one clinical study [251] have investigated the associations and effects of physical activity in premenopausal women.

1. Observational Studies

The earliest of such studies was one of 50 collegiate Japanese premenopausal women, in which physical activity performed during early adolescence, i.e., 13-15 years old, was found to be significantly and inversely associated with follicular (but not luteal) levels of E_2 [245]. Physical activity during late adolescence (16-18 years old) or activity performed in the year prior to the study was not correlated at all with either follicular or luteal levels of E_2. Similarly, follicular and luteal levels of SHBG were not associated with physical activity at any of the life periods assessed by the study. Although this study analyzed samples in both menstrual cycle phases and provided adjustment for age, cycle length, BMI, and birth month, the study sample was very small and no adjustment was made for day of menstrual cycle since no date for the onset of subsequent menstruation was available.
In a much larger cross-sectional study of 636 premenopausal women, vigorous physical activity was significantly associated with lower plasma concentrations of E₂ taken across the menstrual cycle (P = 0.043) [247]. In this study, plasma levels of SHBG and luteal progesterone, however, were not associated with either occupational or vigorous physical activity.

Similarly, a cross-sectional analysis of 565 premenopausal women based on the NHS-II study, found a significant inverse association between moderate-to-vigorous PA and luteal levels of free E₂, E₁, and free testosterone (P = 0.04, 0.01, and 0.02, respectively) [246]. Specifically, women who exercised more than 42 MET-hours/week (equivalent to seven hours of vigorous physical activity) had 14% lower luteal free E₂, 10% lower luteal E₁, and 10% lower luteal free testosterone levels than women exercising less than 3 MET-hours/week (equivalent to one hour of walking). When women with anovulatory and irregular menstrual cycles were excluded from these analyses, the trends became significantly attenuated (8% lower luteal free E₂, 2% lower luteal E₁, and 5% lower luteal free testosterone) and ceased to be statistically significant. Finally, this study reported levels of vigorous physical activity, walking, and sitting/standing were not associated with follicular or luteal plasma levels of E₂, free E₂, E₁, estrone sulfate (E₁SO₄), SHBG, testosterone, free testosterone, and progesterone.

Overall, cross-sectional data consistently suggests higher moderate-to-vigorous physical activity levels in premenopausal women are inversely associated with plasma levels of estrogen and free testosterone but not associated with any other sex hormones or SHBG.
2. Clinical Studies

In comparison, only one of three prospective studies has found an association between physical activity and premenopausal sex hormone levels. In the Tromso Study, a single-center, population-based prospective study in Northern Norway, untimed plasma levels of 205 premenopausal women were not associated with moderate-to-vigorous physical activity [248].

In the Penn Ovarian Aging Study, 391 women provided a follicular sample during two consecutive menstrual cycles at four different assessment periods (baseline, 30 months later, 54 months later, 78 months later) and reported physical activity up to four times over 10 years through the Paffenbarger Physical Activity survey [250]. In this study, leisure-time physical activity of pre- and postmenopausal women was not associated with follicular plasma levels of E\textsubscript{2} and total testosterone. Interestingly, significant associations were only observed in women undergoing transmenopause, that is those experiencing amenorrhea for 3 to 11 months.

In contrast to these two studies, a Polish study of 139 eumenorrheic, premenopausal women found mean salivary E\textsubscript{2} levels collected daily over one menstrual cycle to be significantly and inversely associated with higher habitual physical activity [249]. Women in the highest tertile for habitual physical activity had significantly lower salivary mid-cycle, follicular, and luteal levels of E\textsubscript{2} (\(P = 0.0001\) for all) than women in the lowest tertile. Furthermore, this study observed women in the highest tertile of habitual physical activity spent significantly more time (min/day) in occupational, housework, sport/exercise, and overall daily activities than women in the other tertiles.
Lastly, the strongest evidence on the effects of physical activity on premenopausal estrogen levels comes from a recent, small clinical study of 35 sedentary, eumenorrheic women assigned to either a light conditioning intervention \((n = 9)\) or an exercise intervention coupled with caloric restriction \((n = 24)\)[251]. The exercise prescription consisted of 40-90 minutes of supervised aerobic activity at 80\% of maximum heart rate for four menstrual cycles for 1-2 days a week for the light conditioning (LC) group and four times per week for the exercise/diet group (EX+CR). The diet prescription for the LC group remained eucaloric while that of the EX+CR group targeted a reduction of 20-35\% of baseline energy requirements. Both groups had excellent compliance to the nutrition counseling sessions (95-99\%) and exercise sessions (92-95\%). Blood samples for serum \(^2\)E were collected every 2-3 days totaling ten samples during the baseline cycle and ten samples during intervention cycle 4. Blood samples for SHBG were obtained during follicular days 1-7 for baseline, intervention cycles 1-4, and the post-study cycle. First morning void samples were taken daily during the baseline and intervention cycles and were used to assess estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG).

The total area under the curve (AUC) for \(^2\)E (representing the entire menstrual cycle) at intervention cycle 4 was significantly lower than at baseline in the EX+ CR group \((P = 0.004)\). Similarly, urinary E1G and PdG AUCs across the menstrual cycle for the EX +CR group decreased significantly from baseline values \((P = 0.004\) and 0.011, respectively). The effect of the intervention on SHGB levels in the EX+ CR group showed a biphasic response; SHBG increased significantly by intervention cycle 2 and
then decreased gradually by intervention cycle 4 such that post-intervention levels were not statistically different from baseline levels. In comparison, the LC group experienced no significant changes from baseline in urinary levels of E1G and PdG, or serum levels of E\textsubscript{2} and SHBG.

In conclusion, data in premenopausal women collectively and consistently suggests physical activity, especially of moderate-to-vigorous intensity, has an inverse effect on E\textsubscript{2} and E\textsubscript{1} levels and no effect on SHBG levels. Currently, such a conclusion cannot be extended to testosterone (free or total) or progesterone due to inconclusive or insufficient data.

**B. Estrogen Metabolites**

While there is limited data on the association between physical activity and endogenous sex hormones in premenopausal women, there is an abundance of observational but not experimental evidence in support of an association between physical activity and premenopausal estrogen metabolism.

1. **Observational Studies**

The earliest observations that physical activity led to changes in estrogen metabolism came from small, cross-sectional studies in young female athletes in the mid 1980’s. Specifically, Russell *et al.* observed that both oligomenorrheic competitive swimmers and eumenorrheic runners had significantly higher urinary levels of 2-OHE\textsubscript{1} and lower levels of E\textsubscript{2} at baseline than non-exercising women [252]. In these athletes, levels of 2-OHE\textsubscript{1} were significantly elevated after exercise compared to their pre-exercise levels. In another study, Russell *et al.* reported young competitive swimmers who developed
oligomenorrhea after switching from a moderate to a strenuous training program experienced a significant increase in serum levels of 2-OHE$_1$ (but not E$_2$) [253]. Interestingly, the levels of 2-OHE$_1$ during moderate training were similar to those of non-athlete controls.

Similarly, using radiolabeled tracers, Snow and colleagues showed elite oarswomen with menstrual irregularities metabolized a greater fraction of E$_2$ by 2-hydroxylase oxidation than both oarswomen with normal menstrual function and control women [254]. No significant differences in 2-hydroxylase oxidation were observed between oarswomen with normal menstrual function and controls. The extent of 16α-hydroxylase oxidation, however, remained unchanged and similar in both oarswomen and controls. Collectively, results from these earlier studies suggest higher levels or increased intensity of chronic exercise promotes a shift in estrogen metabolism that favors C2-hydroxylation.

Cross-sectional studies conducted more than two decades later also suggest physical activity may favorably mediate estrogen metabolism in premenopausal women. For instance, a Chinese study found premenopausal women in the highest quartile of leisure-time physical activity (measured in MET per hour/day) had significantly higher urinary 2-OHE$_1$/16α-OHE$_1$ ratio than women in the lowest quartile after adjustment for age, soy protein, and Brassica vegetable intake [255]. Interestingly, a significant positive interaction was observed between 2-OHE$_1$/16α-OHE$_1$ ratio and BMI among the most active women but not among the least physically active.

In another study, regression analyses adjusted for age and BMI showed the average daily MET-hours of physical activity of 77 eumenorrheic premenopausal women to be
significantly associated with higher luteal levels of 2-OHE₁ and 2-OHE₁/16α-OHE₁ ratio but not 16α-OHE₁ levels [256].

The results of another study with a larger sample size (n = 603), better lab methodology (liquid chromatography-tandem mass spectrometry (LC/MS-MS)), timed luteal urine samples, and a previously tested and confirmed reliable physical activity assessment instrument also support the idea that physical activity can mediate estrogen metabolism in premenopausal women [257]. Specifically, high overall physical activity in adulthood (42+ MET-hour/week vs. < 3 MET-hour/week) was associated with a 15% reduction in urinary luteal levels of both E₂ and 2-MeOE₁ and a 24% reduction in 16α-OHE₁ levels after adjusting for age, BMI, alcohol consumption, luteal day at collection, and menstrual cycle length. In addition, high overall physical activity was positively associated with 2-OHE₁/16α-OHE₁, 2-OHE/parent estrogens, and 2-OHE/2-MeOE ratios. Significant associations were also observed for walking and vigorous physical activity in adulthood, but there was little evidence of an association with adolescent physical activity or sedentary behavior in adolescence or adulthood.

In contrast, a study in which aerobic fitness was used as an objective indicator of chronic physical activity, follicular and luteal levels of 2-OHE₁, 16α-OHE₁, and 2-OHE₁/16α-OHE₁ ratio of highly fit premenopausal women (maximum oxygen consumption (VO₂ max) ≥ 48mL.kg.min⁻¹) were found to be similar to those of average fit women (VO₂ max ≤ 40 mL.kg.min⁻¹) [258].
Overall, observational studies highly suggest physical activity, whether as purposeful exercise or recreational activity, can mediate changes in estrogen metabolism of premenopausal women.

2. Clinical Studies

The first experimental studies in premenopausal women initially focused on the effects of acute not chronic exercise on estrogen metabolism. The knowledge derived from these studies is almost due entirely to the work of Carl De Cree and colleagues in the late 1990’s. In one of many studies, De Cree and colleagues studied nine normally menstruating, untrained young women who underwent a standardized incremental exercise test during the follicular and the luteal phase [259]. Results from this study showed baseline serum levels of total estrogens (E), 2-hydroxyestrogens, and 2-methoxyestrogens to be significantly higher in the luteal phase than in the follicular phase. However, with the exception of total E (which only increased significantly in the luteal phase), incremental exercise intensity did not result in significant changes in total 2-OHE, total 2-MeOE, catechol estrogen (CE) formation (expressed as 2-OHE/E ratio), or CE activity (expressed as 2-MeOE/2-OHE ratio) in either menstrual phase [259].

In a subsequent study, nine untrained eumenorrheic women were tested in the follicular and luteal phase of a control (pre-training) cycle and also after a 5-day exhaustive training program. Similar to their previous study, De Cree et al. confirmed total plasma levels of E, 2-OHE, and 2-MeOE were significantly higher in the luteal phase than in the follicular phase [260]. Compared to pre-training levels, luteal post-
training levels of E, 2-OHE, and 2-MeOE were significantly lower while CE formation and CE activity were significantly higher.

In a third study, nine healthy, eumenorrheic, untrained women underwent a pre- and post-training incremental exercise test during the follicular and luteal phase [261]. Results showed follicular and luteal levels of E decreased significantly after five days of intense training compared to pre-training levels while exercise at all intensities increased estrogen levels significantly from those at rest. In the case of 2-OHE and 2-MeOE, plasma post-training levels decreased significantly compared to pre-training levels regardless of intensity but only during the luteal phase, especially for 2-OHE. Conversely, CE formation and CE activity in the luteal phase increased significantly from pre-training levels while decreasing with exercise intensity.

While at face value the results reported by De Cree and colleagues (i.e., exercise induces CE formation) and those by Russell et al. (i.e., exercise increases 2-OHE plasma levels) seem to contradict each other, the results can be reconciled when one calculates the CE formation in the studies of the latter. In this manner, De Cree and colleagues found the CE formation in Russell’s controls to be 0.13 ± 0.03; 0.77 ± 0.30 in eumenorrheic swimmers training 55 km/week; and 1.20 ± 0.21 in oligomenorrheic swimmers training 90 km/week [261]. Based on these calculations, De Cree and colleagues speculate that as long as a subject is euestrogenemic, a higher formation of CE may not be accompanied by increases in absolute levels of CE. One obvious argument against this conclusion however, is that it is based on data obtained by indirect methods,
that is, CE formation was calculated arithmetically by dividing the concentration of plasma 2-OHE levels by E levels.

In another study, De Cree et al. was able to address this criticism indirectly by measuring O-methylation of 2-hydroxyestrogens directly in human erythrocytes. This method has been shown to be an accurate measure of COMT activity in other tissues and significant for the \textit{in vivo} methylation rate of CE [262]. Therefore, a direct method for measuring CE activity (not CE formation) was used. In this study, levels of plasma 2-MeOE and 4-MeOE of 15 healthy, eumenorrheic untrained women following similar training protocols as in the previous studies increased significantly after training in both menstrual phases [263]. However, the effect of incremental exercise was only apparent in the formation of 4-MeOE, especially in the luteal phase.

In a later study and consistent with previous results, De Cree et al. showed plasma levels of 4-OHE and 4-MeOE of untrained, healthy, eumenorrheic women increased with incremental exercise [264]. Specifically, 4-OHE levels increased substantially more in the pre-training state while 4-MeOE levels increased more so after training. Furthermore, the ratio of 4-MeOE to 4-OHE, a measure of CE activity, increased significantly with progressive training.

Collectively, the work of De Cree and colleagues suggest acute exercise in eumenorrheic women shifts estrogen metabolism in favor of 2- vs. 16\(\alpha\)-hydroxylation and CE activity through increased COMT activity leading to higher levels of 2- and 4-methoxyestrogens.
While data on the effect of acute exercise is valuable, it has limited application to the general female population, as women do not engage in enough physical activity and/or high intensity exercise as the athletes or women in these acute exercise studies did. In that regard, current studies have focused on the chronic effect of usual, recreational physical activity on estrogen metabolism. To date, five exercise intervention studies [265-269] have investigated the effects of chronic aerobic exercise on the estrogen metabolism of premenopausal women.

In the Women’s Healthy Lifestyle Project study, 84 healthy premenopausal women followed a diet and exercise intervention for six months while 90 women were randomized into an assessment-only control group [266]. The diet/exercise intervention was designed to induce a 5- to 15-lb weight loss by decreasing dietary cholesterol to ≤ 100 mg/day, limiting total fat and saturated fat intake to ≤ 25% and ≤ 7% of calories, respectively; and increasing moderately intensity physical activity. In the treatment group, the intervention resulted in a significant increase in urinary 2-OHE$_1$/16α-OHE$_1$ ratio compared to baseline values. The control group, however, also experienced (inexplicably) a positive change from baseline values. Ultimately, the difference between groups in the change from baseline in 2-OHE$_1$/16α-OHE$_1$ ratio was not statistically different. Limitations of this study include untimed spot urine samples, retrospective assessment of physical activity through a questionnaire, and unexplained observation of a significant increase in 2-OHE$_1$/16α-OHE$_1$ ratio in the control group.

In another diet and exercise study, 24 eumenorrheic premenopausal women followed an exercise intervention lasting four menstrual cycles seeking to increase caloric
expenditure by 20% while also reducing daily caloric intake by 20-35% [269]. Subjects had supervised exercise sessions four times per week during which they exercised aerobically on a treadmill, stationary bike, or stair stepper for approximately 40-90 minutes at 60-90% of their maximum heart rate (max HR). Additionally, subjects were instructed to track their daily food consumption for seven days at a time, every two weeks, and review these records every other week with a study dietitian. Estrogen metabolite values were assessed via EIA from first morning voids collected daily during a one-month baseline cycle and the subsequent four intervention cycles. Women in the study showed high adherence to the exercise protocol (96% compliance) and diet intervention (22% reduction in daily caloric intake) resulting in significant improvements in aerobic fitness and significant decreases in body weight (3.8 kg) and body fat percentage (4.6%). In addition, levels of $2\text{-OHE}_1$ and $16\alpha\text{-OHE}_1$ were reported to be significantly higher in the luteal phase compared to the follicular phase. However, urinary levels of $2\text{-OHE}_1$ and $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio did not change significantly over the course of the study or between menstrual cycle phases, while levels of luteal $16\alpha\text{-OHE}_1$ increased significantly in a non-linear (cubic) fashion.

Interestingly, when women in this study were divided into tertiles according to their baseline $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio, those in the lowest tertile experienced the greatest percent changes (increases) in follicular and luteal $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio and absolute changes in the luteal phase that women in the second and third tertiles did. Importantly, women in the lowest tertile did not differ from women in the other tertiles in baseline weight, percent body fat, or aerobic fitness, or in the amount of weight or fat loss and
improvement in aerobic fitness during the intervention suggesting baseline $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio could be a major determinant of changes in $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio. The study design, however, makes it impossible to determine whether the significant changes in $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio were due to the exercise intervention, calorie restriction, weight/fat loss, or a combination of these factors.

In an exercise-only intervention, 15 young, eumenorrheic, sedentary, premenopausal women exercised aerobically at an incrementally higher intensity (70% max HR to 85% max HR) for 30 minutes on a treadmill or elliptical machine five times a week, for three to four menstrual cycles [268]. During the study, subjects were asked to avoid changes in diet or weight and to collect their urine for three consecutive 24-hr periods corresponding to menstrual cycle days 7, 8, and 9 of a pre- and post-intervention cycle. Urine was pooled and analyzed as a single sample via modified gas chromatography-tandem mass spectrometry (GC-MS/MS). Results from this study show that approximately 15 weeks of moderate-to-vigorous intensity aerobic exercise in premenopausal women did not result in statistically significant changes from baseline on follicular levels of $E_1$, $E_2$, $2\text{-OHE}$, $4\text{-OHE}$, $16\alpha\text{-OHE}_1$, $E_3$, $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ ratio and $2\text{-OHE}/4\text{-OHE}$ ratio.

In a similarly small but much longer exercise-only intervention, 20 young, sedentary premenopausal women were asked to exercise aerobically at 60-70% of their max HR for at least 60 minutes three days a week for six months [267]. First morning urine was collected on menstrual cycle day 3 and also before and after the afternoon exercise program and analyzed via GC/MS-MS. Urinary follicular levels of $E_1$, $E_2$, and $E_3$ did not change significantly after the intervention when compared to pre-intervention levels.
To date, only one randomized exercise-controlled clinical trial in premenopausal women has been conducted. This study also failed to find significant changes in estrogen metabolism due to exercise. Specifically, Campbell et al. recruited and randomized 32 young, sedentary, healthy, eumenorrheic women into either a 12-week aerobic exercise program (n = 14) or a usual lifestyle intervention (n = 15) [265]. Randomization was stratified on BMI (< 25 or ≥ 25) and occurred once baseline measurements were obtained during a control cycle.

During weeks 1-4, exercise participants trained aerobically for 20-40 minutes on a stationary bike three times a week at an intensity of approximately 25% higher than the power output recorded at a baseline fitness assessment. During weeks 5-8, participants did two aerobic sessions for 30-45 minutes as in weeks 1-4 and two 40-minute session of interval training. Finally, during weeks 9 to 12, participants did two base aerobic training sessions for 30-45 minutes and two interval sessions similar to weeks 5 to 8.

First morning urine samples were collected during menstrual cycle days 20 and 22 over four consecutive menstrual cycles (one baseline and three intervention cycles) and analyzed via EIA methods.

At baseline, there were no differences in the luteal levels of 2-OHE$_1$, 16α-OHE$_1$, and 2-OHE$_1$/16α-OHE$_1$ ratio between exercisers and control women. Similarly, there were no significant differences within- and between-groups in the levels of 2-OHE$_1$, 16α-OHE$_1$, and 2-OHE$_1$/16α-OHE$_1$ ratio due to the exercise intervention. While the intervention resulted in no changes in body weight, BMI, waist and hip circumference, exercise participants lost a significant amount of body fat (1.2 kg) and gained lean mass (0.9 kg).
when compared to control women. Lean body mass was found to be positively and significantly associated with the 2-OHE\textsubscript{1}/16\textalpha-OHE\textsubscript{1} ratio ($r = 0.43; P = 0.015$).

Although the exercise intervention by Campbell et al. used a robust study design, it does have some limitations that could have compromised their ability to detect significant changes on estrogen metabolism including small sample size (14-15 women per group) and difficulty standardizing urine sample collection across the menstrual cycle.

Overall, exercise interventions in premenopausal women (whether coupled with a diet intervention or not) do not support the hypothesis that aerobic exercise is capable of inducing significant changes in estrogen metabolism.

The discrepancy observed between observational studies and exercise interventions may be due in part to the inherent limitations of the observational design and methodology. For instance, in the earlier cross-sectional studies [252-254], sample sizes were very small (5-7 participants per group), menstrual function was not standardized (comparison between eumenorrheic and oligomenorrheic women), reproductive age among subjects varied widely, physical activity was assessed via self-reported measures, and lab methods used an older version of radioimmunoassay [252, 253] or administration of radiolabeled tracer [254] to measure estrogen metabolites, which are not as accurate as the newer solid-phase EIA [107]. In contrast, the more recent studies [255-258] have used larger sample sizes, adjusted or controlled for menstrual cycle phase, and used an improved [270] EIA method [255, 256, 258] or GC-MS/MS methodology [257]. With the exception of one study [258], all of the recent observational studies [255-257] have also
relied on self-reports of physical activity, which are susceptible to recall bias and over reporting of frequency, duration, and intensity [271].

Future exercise interventions should use a control group, be designed to have enough statistical power to detect a minimum difference in estrogen metabolite concentrations, account for menstrual cycle differences, and expand the scope of estrogen metabolite analyses to include the 4-hydroxyestrogens, 2- and 4-methoxyestrogens and their metabolite ratios.

V. DISSERTATION RATIONALE

Most risk factors for breast cancer are not or not easily modifiable. Physical inactivity is one of few risk factors that are reasonably amenable for intervention, and as a result, it has become a primary target for behavior modification in breast cancer prevention research.

Cohort and case-control studies have been instrumental in providing compelling evidence of an inverse association between physical activity and breast cancer risk. Observational data, however, has important limitations. First of all, the use of self-reported, mostly retrospectively, physical activity is subject to recall bias. Secondly, and most importantly, observational studies are not designed to determine the exact biological mechanisms by which physical activity (and its optimal modality, intensity, frequency, duration) mediate breast cancer risk. In this regard, an exercise randomized clinical trial is necessary to address both of these issues.
The doctoral project presented in this dissertation used some of the data generated by the Women In Steady Exercise Research (WISER) study conducted at the University of Minnesota. The WISER study is the first randomized, exercise-controlled, clinical trial investigating the effects of aerobic exercise (30 minutes a day, five times a week for four menstrual cycles) on a myriad of breast cancer risk biomarkers of sedentary, healthy, premenopausal women. Circulating levels of F₂-isoprostanes, IGF-1, IGFBP-1, IGFBP-2, and IGFBP-3, insulin, fasting glucose, endogenous sex hormones, estrogen metabolite excretion, body composition parameters, and dietary intake were measured both at baseline and post-intervention. Based on the results of a pilot study [268], 4 menstrual cycles (average of 16 weeks) was the minimum amount of weight-bearing aerobic exercise needed to observe significant changes in the levels of the aforementioned breast cancer biomarkers. In addition, the specific exercise protocol sought to follow the current public health recommendations by the American College of Sports Medicine to promote and maintain health and prevent chronic disease and that of the International Agency for Research in Cancer to prevent breast cancer.

In particular, this doctoral project sought to test the hypothesis that 150 minutes of moderate-to-vigorous aerobic physical activity per week for 16 weeks would reduce circulating levels of sex hormones and shift estrogen metabolism in premenopausal women in a matter consistent with decreased breast cancer risk. The specific hormonal endpoints analyzed include serum levels of E₂, E₁SO₄, progesterone, testosterone, and SHBG via RIA and ELISA methods and urinary levels of parent estrogens and ten
estrogen metabolites (2-OHE$_1$, 2-OHE$_2$, 4-OHE$_1$, 4-OHE$_2$, 16a-OHE$_1$, E$_3$, 2-MeOE$_1$, 2-MeOE$_2$, 4-MeOE$_1$, and 4-MeOE$_2$) via LC/MS-MS.

The study intervention focused exclusively on premenopausal women to address the possibility that physiological changes associated with breast cancer may take place before menopause. This is important to consider given it is well accepted that cancer initiation and its development may occur over a long period of time.

The WISER study was designed to address many of the shortcomings of previous exercise interventions in premenopausal women, which are currently very few. For instance, the sample size of the WISER study ($n = 320$) is roughly an order of magnitude greater than other studies. Secondly, confounding effects were optimally minimized by the use of a control group chosen at random. Thirdly, the WISER study was designed to analyze a much broader hormonal profile using a more accurate and sensitive lab methodology than the one chosen by previous studies.

Given these methodological advantages, the WISER study is better equipped to address the scientific question of whether or not the hormonal changes (if any) associated with aerobic exercise are consistent with those deemed to decrease breast cancer risk in premenopausal women.
Figure 1.1. Steroidogenic Pathway in the Ovary. Abbreviations: StAR, steroidogenic acute regulatory protein; CYP11A1, side-chain cleavage of P450; CYP17A1, 17-hydroxylase/17,20-lyase; HSD3B2, 3β-hydroxysteroid dehydrogenase-Δ5,4 isomerase type 2; CYP19A1, aromatase; HSD17B1, 17β-hydroxysteroid dehydrogenase type 1 [32].
CHAPTER 2:

ENDOGENOUS SEX HORMONES AND SHBG
I. SUMMARY

Background. It is hypothesized that exercise can lead to a decrease in breast cancer risk through several hormonal and non-hormonal mechanisms. The Women in Steady Exercise Research (WISER) study investigated the effects of aerobic exercise on premenopausal sex hormone levels.

Methods. 391 sedentary, healthy, young eumenorrheic women were randomized into an exercise intervention of 30 minutes of aerobic exercise 5 times a week for approximately 16 weeks ($n = 212$), or into a control group ($n = 179$). Serum levels of estradiol, estrone sulfate, testosterone, and sex hormone binding globulin (SHBG), all in the midfollicular phase, and of progesterone, in the midluteal phase, were measured at baseline and at the end of the 16-week period.

Results. Compared to the controls ($n = 153$), exercisers ($n = 166$) experienced significant increases in aerobic fitness, lean body mass, and decreases in percent body fat. There were no significant changes in body weight and menstrual cycle length between or within groups. Progesterone decreased significantly in exercisers; however, this reduction was similar to that of the control group. No significant changes between or within groups were found for any of the other sex hormones or SHBG.

Conclusions. In premenopausal women, 16 weeks of 150 minutes per week of moderate aerobic exercise in young women did not significantly alter sex hormone or SHBG levels.

Impact. Any favorable effects that moderate aerobic exercise without an associated weight change may have on breast cancer risk in premenopausal women are unlikely to be a consequence of changes in levels of sex hormones or SHBG.
II. INTRODUCTION

Despite steady decreases in breast cancer mortality rates, breast cancer continues to be the most frequently diagnosed non-skin cancer and second leading cause of cancer death among women[272]. Well-established risk factors for breast cancer include early age at menarche, late age at menopause and first childbirth, nulliparity, family history of breast cancer, benign breast disease, and non-reproductive factors such as hormone-replacement therapy use and physical inactivity [273, 274]. Collectively, these factors increase the lifetime exposure of breast tissue to circulating sex hormones, which have been implicated, both experimentally and observationally, in the etiology of breast cancer.

In cultured mammary cancer cells, estrogen has been shown to promote cell proliferation [86, 275]. Furthermore, although the role of progesterone in breast cancer is unclear, there is evidence progesterone can potentiate the mitogenic effect of estradiol [276]. Although not a steroid hormone, the glycoprotein sex hormone binding globulin (SHBG) is also thought to play a role in breast carcinogenesis not only by regulating the bioavailability of estradiol and testosterone in circulation [277], but also by inhibiting estradiol-mediated cell growth and anti-apoptosis in estrogen-dependent breast cancer cells [278].

In observational studies, elevated levels of circulating sex hormones are strongly associated with decreased risk for breast cancer. In a re-analysis of 13 prospective studies, postmenopausal women with the highest levels of estradiol, estrone, and testosterone had a two-fold increase in breast cancer risk, and those with the highest
SHBG levels had a 34% decreased risk [279]. Similarly, studies in premenopausal women have shown associations between increases in breast cancer risk with higher levels of estrogens and androgens and lower levels of progesterone and SHBG [60, 62, 63, 65, 66, 69, 75, 280-284]. Importantly, premenopausal levels of estradiol have been associated with postmenopausal breast cancer [65], suggesting exposures during this period may very well play a role in the initiation and promotion of breast cancer.

Physical activity is a modifiable lifestyle that has been associated with reductions in breast cancer risk of approximately 25-30% [285]. Although many mechanisms have been suggested for the protective effect of exercise on breast cancer, reduction of circulating levels of sex steroid hormones is one that has been widely suggested [286]. This has not been substantially studied in clinical trials, although in a recent, small clinical study of sedentary premenopausal women reported by Williams et al. [287], a four-cycle intervention consisting of moderate-intensity aerobic exercise in combination with a caloric restrictive diet resulted in significant decreases in serum estradiol and urinary estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG) levels.

In contrast, our study, the Women In Steady Exercise Research (WISER) study, was a randomized trial of premenopausal women that investigated the effects of a moderate-to-vigorous exercise intervention independent of diet restriction and weight loss. We specifically sought to determine if the exercise intervention would lead to alterations in levels of sex hormones and SHBG that would be consistent with a decreased risk of breast cancer.
III. MATERIALS AND METHODS

The WISER study was a randomized, controlled, parallel-arm study that investigated the effects of a 16-week, moderate-to-vigorous intensity, aerobic exercise intervention on breast cancer biomarkers in young, healthy, sedentary, eumenorrheic women. The study was approved by the Human Subjects Review Committee at the University of Minnesota (Institutional Review Board; IRB ID#0505M69867). Written informed consent was obtained from each participant prior to participation. Details of the study design and methods have been described previously [288].

Briefly, 391 non-smoking women aged 18-30 years residing in the Minneapolis-St. Paul metropolitan area with a body mass index (BMI) of 18-40 kg/m^2 (inclusive), having a self-reported menstrual cycle length of 24 to 35 days and a sedentary lifestyle (two or less weekly sessions of moderate intensity exercise) were randomized into the WISER study. Exclusion criteria included use of hormonal contraceptives in the past three months of any form or depot-medroxyprogesterone acetate in the past 12 months, gynecological problems, metabolic or endocrine-related diseases, non-melanoma cancer in the past 5 years, alcohol consumption of > 7 servings per week, current or recent (past 6 months) pregnancy, and body weight changes greater than 10% over the past year. A total of 391 women started the study by completing baseline measurements during the luteal phase of menstrual cycle 1 and the follicular phase of cycle 2.

Randomization to either an exercise intervention or a no-exercise control group occurred after both baseline measurements were taken. Women with menstrual cycles averaging 25-31 days concluded the study with follow-up measurements during the luteal
phase of cycle 5 and the follicular phase of cycle 6. Women with menstrual cycle lengths outside this range had follow-up measurements scheduled such that study duration after randomization was no less than 14 weeks and no more than 18 weeks. Specifically, women with menstrual cycle lengths of less than 25 days provided follow-up measurements during the luteal phase of menstrual cycle 6 and the follicular phase of cycle 7, while women with menstrual cycle lengths of more than 31 days completed these measurements during the follicular and luteal phases of cycle 5.

Randomization was stratified on baseline BMI tertiles ($\leq 22.8$, 22.8-26.3, $\geq 26.3$) based on the 50th and 75th percentiles from NHANES I data and age (18-24 vs. 25-30). Initially, the randomization ratio (exercise:control) was 1:1 but due to the higher dropout rate in the treatment group it was later changed to 60:40 to ensure adequate sample size in both groups was achieved within the projected study timeline. While failure to return for follow-up measures resulted in being dropped out of the study, exercisers were additionally subject to study exclusion if they missed 15 or more exercise sessions. Figure 2.1 shows the screening, randomization, retention, and completion of WISER participants.

**Exercise Intervention**

Women randomized to the exercise intervention trained aerobically five times a week for 30 minutes on a treadmill, stair-stepper, or elliptical machine, at a specified intensity based on age-predicted maximal heart rate (max HR) for 4 menstrual cycles (14-18 weeks). All training sessions took place at the University of Minnesota’s Recreation Center. However, under special circumstances (housing relocation, time constrains, or
traveling issues) participants were allowed to work out at another exercise facility. The exercise intensity was initially set at 65%-70% of the age-predicted max HR and was gradually increased by 5% every four weeks until 80%-85% of age-predicted max HR was reached (stage 1 = 65-70%; stage 2 = 70-75%; stage 3 = 75-80%; stage 4 = 80-85%).

A certified personal trainer provided instruction on how to properly use the exercise machines and thoroughly complete an exercise log after each workout. Trainers supervised exercise sessions and reviewed the exercise logs at least once weekly to monitor adherence and safety. When not meeting with a trainer, participants were expected to complete the remaining of the workout sessions on their own at the specified training facility. Exercise adherence was monitored by a heart rate monitor (Polar Electro Inc., Lake Success, NY) and exercise logs. When the trainer detected a missed exercise session in the exercise logs, she contacted the participant to determine the reason for the missed session and to encourage compliance with the study protocol. Any physical activity performed after randomization and outside the prescribed exercise intervention was assessed with a physical activity questionnaire by a research member at the end of the study.

All participants, regardless of randomization outcome, were advised to maintain their baseline body weight. Control participants were asked to maintain their usual level of physical activity and to not change their eating habits. A thorough description of the exercise intervention has been described previously [288].
**Outcome Measures**

All biological, anthropometric, and body composition measures were taken at the General Clinical Research Center at the University of Minnesota. Body weight was measured four times during the study (baseline, cycle 3, cycle 4, and at follow up) to the nearest 0.1 kg, using an electronic scale (Scale Tronix, White Plains, NY). Height was measured by a stadiometer at baseline without shoes to the nearest 0.1 cm (Scale Tronix, White Plains, NY). Body mass index was calculated by dividing weight in kg by height in meters squared ($\text{kg/m}^2$). Body composition was assessed at baseline and follow-up luteal phase clinic visits by dual energy x-ray absorptiometry (DXA) using a Lunar Prodigy DXA apparatus (Lunar Radiation Corp., Madison, WI).

A sub-maximal treadmill test was used to assess aerobic fitness at baseline and immediately after the intervention. This workload was then converted to metabolic equivalents (METs) by using a standard conversion formula [289]. Details of the fitness protocol have been described previously [288]. Self-reported physical activity performed a year prior to the study and during the 4-month follow-up period was assessed by research staff via a modified version of the Modifiable Activity Questionnaire [290]. This information was transformed into MET-hours per week (MET-hrs/wk) using commonly accepted MET values [291]. Dietary intake was assessed through self-reported, 3-day food records at baseline and follow-up. Nutrient intake was determined using The Food Processor SQL® by ESHA Research (Salem, OR).

Timing and occurrence of ovulation was assessed using a commercial 9-day Assure LH™ ovulation kit (Conception Technologies, San Diego, CA). This kit assesses for
luteinizing hormone (LH) surge via ELISA, with a 96% accuracy rate with home use. For purposes of the study, day of ovulation was considered to be the day after a positive LH surge result. Participants were asked to inform research staff of positive LH surge results each month either by email or phone.

**Hormone and SHBG Analysis**

Blood samples were drawn between 6:45 and 11:00 am after an overnight fast, centrifuged for 15 minutes (4°C at 1000 x g); serum was separated, aliquoted, and stored frozen at -70°C. Baseline and follow-up blood draws took place during specific days of the menstrual cycles. Midluteal phase blood draws were scheduled 6-9 days after ovulation for analysis of progesterone during cycles 1 and 5 (cycle 6 for women with menstrual cycle lengths of less than 25 days) while midfollicular phase blood draws were on cycle days 7-10 for the analysis of all other sex hormones and SHBG during cycles 2 and 6 (cycle 5 and cycle 7 for women with menstrual cycle lengths of less than 25 days and more than 31 days, respectively).

Serum concentrations of estradiol, estrone sulfate, testosterone, progesterone, and SHBG were measured by laboratory personnel blinded to the intervention status. Commercially available RIA kits (Diagnostic System Laboratories, Webster, TX) were used to measure estradiol (DSL-4400), estrone sulfate (DSL-5400), testosterone (DSL-4100), and progesterone (DSL-3900). An ELISA method (Immuno-Biological Laboratories-America, Minneapolis, MN) was used to measure SHBG (IBL-59106). Free and bioavailable fractions of estradiol and testosterone were calculated using the equations by Vermeulen *et al.* [292] and association constants estimated by Mazer [293].
Although not a sex hormone, SHBG will be referred as such in the remainder of the text for ease of expression.

Samples were assayed in duplicate and in batches such that each batch contained both baseline and follow-up samples from each participant and an equal number of exercise and control participants. Two quality control blood samples were included in each batch. The mean intra-assay and inter-assay CVs were 5.7% and 16.6% for estradiol; 3.7% and 12.7% for estrone sulfate; 6.1 and 23.2% for testosterone; 8.6% and 11.7% for progesterone; and 4.9 and 5.2% for SHBG.

**Statistical Analysis**

Unadjusted comparisons of baseline characteristics were performed by using Student’s *t*-tests for continuous variables and chi-square tests for categorical variables. Baseline associations between sex hormones and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were determined using Spearman correlation coefficients. The main trial analysis assessed the intervention effect on hormones on an intent-to-treat basis such that all samples from participants who completed at least one follow-up blood measure were included in the analysis regardless of compliance level. Comparison of sex hormone levels at baseline, follow-up, and changes from baseline were adjusted for age and BMI strata with a general linear model. Baseline and follow-up analyses were conducted using log transformed hormone values while changes from baseline were analyzed on the original scale. Linear models were calculated using SAS software, version 9.2 (SAS institute Inc., 2008, Cary, NC). *P* < 0.05 was considered statistically significant.
IV. RESULTS

Study participants

As shown in Figure 1, of the 212 and 179 women randomized into the exercise and control groups, 166 (78.3%) and 153 (85.5%), respectively, completed the WISER study ($P = 0.68$). With the exception of education level ($P = 0.10$), women who dropped out of the study were no different than women who completed the study in terms of age, height, weight, BMI, race, ethnicity, marital status, previous contraceptive use, and parity (data not shown). Both baseline and follow-up midfollicular and midluteal phase samples were obtained from 319 and 311 women, respectively. Most of the women who completed the study were single (82%), Caucasian (72%), educated (67% were at college level or higher), and had a normal BMI (63.5%). There were no significant differences in baseline demographic characteristics between the study groups (Table 2.1).

Baseline associations

At baseline, estradiol was significantly associated with assessed fitness ($r = -0.14, P = 0.01$). Testosterone was significantly correlated with age ($r = -0.15, P = 0.008$), BMI ($r = 0.13, P = 0.02$), and percent body fat ($r = 0.16, P = 0.005$). Progesterone was significantly associated with age ($r = 0.12, P = 0.03$). SHBG was positively associated with age ($r = 0.11, P = 0.05$) and negatively associated with BMI ($r = -0.22, P < 0.001$) and percent body fat ($r = -0.21, P = 0.001$). There were no significant associations between any of the sex hormones and self-reported physical activity, energy or alcohol intake, or reproductive factors such as age at menarche.
**Treatment adherence**

Adherence to the exercise intervention in the WISER study was excellent; on average, exercise participants completed 134 minutes per week of the assigned 150 minutes exercise intervention. Exercise adherence in stage 1 was 97.6% and 85.3% in stage 4. More details about the exercise adherence can be found somewhere else [294].

**Treatment effects**

The exercise intervention resulted in a significant increase in aerobic fitness (increase of 0.90 METs reached at 85% of max HR for exercisers vs. 0.12 METs for controls) and improvements in body composition measures. Exercisers gained more lean mass (0.55 kg vs. 0.07 kg) and lost significantly more fat mass (0.57 kg vs. 0.04 kg) and body fat (0.95% vs. 0.09%) than controls. No changes in body weight were observed in either group (Table 2.2).

There were no differences between exercise and control groups in both baseline and follow-up sex hormone or SHBG levels, except that exercisers had significantly lower estrone sulfate, with and without adjustment for baseline levels (Table 2.3). Similarly, adjustment for baseline levels to changes from baseline comparisons in estrone sulfate resulted in similar means and $P$ values as those obtained without the adjustment. Therefore, the results reported in Table 2.3 for follow-up and changes from baseline in estrone sulfate are age- and BMI-adjusted only.

With the exception of progesterone, there were no differences within-group in sex hormone or SHBG levels. Progesterone levels decreased modestly but significantly ($P = 0.02$) in exercisers; however, this reduction was statistically similar to that experienced
by control participants. No differences were found between groups in change from baseline in any of the sex hormones or SHBG. Results were consistent when comparisons were restricted to normal weight, overweight, and obese subgroups. There were no significant changes from baseline in menstrual cycle length between or within groups (data not shown).

V. DISCUSSION

According to the American College of Sports Medicine and the American Heart Association, 30 minutes of moderate-intensity aerobic exercise performed five times a week is consistent with the promotion and maintenance of health [295]. The WISER study was the first randomized, controlled study designed to test whether a moderate-to-vigorous exercise regimen resulting in no weight loss would result in changes in circulating levels of blood sex hormones and SHBG associated with reduction of breast cancer risk in premenopausal women.

The exercise intervention in the WISER study resulted in favorable changes in aerobic fitness and body composition measures; however, no significant differences were observed between exercisers and control participants in the changes of serum estradiol, estrone sulfate, testosterone, progesterone, or SHBG. Although the study was not specifically powered to assess for hormonal differences between the two groups, the virtually identical results for these parameters make it unlikely that physiologically important differences were present. Most cross-sectional data are consistent with these null results. For example, previous studies have found no significant associations
between physical activity and premenopausal levels of estrone sulfate [248], testosterone [246, 250], progesterone [246, 247], and SHBG [245-247]. As for total and free estradiol, only two [247, 249] of six [245-250] and one [246] of two [246, 248] studies, respectively, have found a significant negative association. In contrast to our results, the one study that evaluated the association between physical activity and both estrone and free testosterone did find significant negative associations [246].

More importantly, our results are consistent with three small clinical studies in premenopausal women. In a study by Rogol et al. [296], there were no differences in integrated estradiol and progesterone levels in seventeen subjects who completed one year of endurance training compared to six (nonrandomized) controls. In the WISER pilot study, 15 weeks of moderate-to-vigorous intensity aerobic exercise in fifteen sedentary premenopausal women resulted in no significant changes in urinary estradiol or estrone levels despite a significant, albeit small (1.2 kg) loss in weight [268]. In the study of Williams et al., sedentary premenopausal women allocated to 120-240 minutes per week of moderate exercise in combination with a caloric restrictive diet (20-35% of baseline energy requirements) experienced significant reductions in body weight (3.7 kg), serum estradiol, and urinary EIG and PdG levels [287]. However, the control participants, who followed an intervention comparable to that of the WISER exercisers (36 minutes of moderate exercise twice a week in addition to unrestrictive, eucaloric diet), did not experience significant changes in body weight or sex steroid levels.

There are different reasons why the exercise intervention of the WISER study may have failed to have resulted in detectable changes in sex hormone and SHBG levels. It
has been hypothesized that the effects of exercise on reproductive hormones are mediated by changes in body composition [285, 297]. While such changes are less important for cycling premenopausal women as compared to postmenopausal women, in whom the primary source of estrogens is peripheral aromatization of androgens in adipose tissue [298, 299], in our study the lack of any such effects may have been because body composition changes were modest. Thus perhaps a longer exercise intervention would have yielded different results. It is also possible that a more intensive sampling and/or sampling closer to the time of ovulation (when estradiol levels are higher) would have improved our ability to detect changes in hormone concentrations.

Alternatively, it is possible exercise exerts an independent effect on hormone exposure by disrupting hypothalamic function resulting in changes in menstrual cycle characteristics such as delayed onset of menarche, irregular or absent menstrual periods, abnormal or loss of luteal function, and longer menstrual cycle length [285, 297]. In our study, although we observed no significant within- or between-group changes in menstrual cycle length, it is possible follicular and luteal phase lengths may have changed significantly even when no changes in menstrual cycle length were detected. We are currently investigating whether the WISER exercise intervention resulted in changes in follicular and luteal phase lengths as well as changes in estrogen metabolism.

Finally, it is possible exercise may decrease breast cancer risk in premenopausal women through non-hormonal mechanisms such as changes in endogenous oxidative stress, insulin and glucose metabolism, inflammatory marker levels, and immune
function [285, 297]. We plan to separately report the effects of the exercise intervention on biomarkers for these mechanisms.

The WISER study is the first clinical trial with randomized controls to study the effects of aerobic exercise on serum reproductive hormone levels in premenopausal women. Strengths include a large sample size, carefully timed follicular and luteal blood samples, and excellent protocol adherence. Findings from this study do not support the hypothesis that, at least in the absence of weight change or obvious menstrual cycle disruption, 150 minutes per week of moderate aerobic exercise leads to reductions in sex hormone concentrations and increases in SHBG concentrations in premenopausal women.
Table 2.1: Baseline Characteristics of Randomized Participants (n = 319) by Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>Exercisers (n = 166)</th>
<th>Controls (n = 153)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.4 ± 3.4</td>
<td>25.2 ± 3.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.9 ± 6.9</td>
<td>165.4 ± 7.5</td>
<td>0.54</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.4 ± 14.6</td>
<td>67.6 ± 14.6</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 4.7</td>
<td>24.7 ± 4.8</td>
<td>0.88</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>24.2 ± 11.2</td>
<td>24.1 ± 10.6</td>
<td>0.90</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>36.4 ± 8.8</td>
<td>36.1 ± 8.3</td>
<td>0.77</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>39.7 ± 5.0</td>
<td>40.0 ± 5.2</td>
<td>0.63</td>
</tr>
<tr>
<td>Weight Categories</td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Underweight (BMI &lt; 18.5)</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5 ≤ BMI &lt; 25)</td>
<td>100 (60%)</td>
<td>100 (65%)</td>
<td></td>
</tr>
<tr>
<td>Overweight (25 ≤ BMI &lt; 30)</td>
<td>46 (28%)</td>
<td>30 (20%)</td>
<td></td>
</tr>
<tr>
<td>Obese (BMI &gt; 30)</td>
<td>19 (11%)</td>
<td>22 (14%)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>White</td>
<td>124 (75%)</td>
<td>107 (70%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>13 (8%)</td>
<td>12 (8%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>20 (12%)</td>
<td>26 (17%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9 (5%)</td>
<td>8 (5%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>8 (5%)</td>
<td>6 (4%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>High school or less</td>
<td>11 (7%)</td>
<td>7 (4%)</td>
<td></td>
</tr>
<tr>
<td>Some college</td>
<td>43 (26%)</td>
<td>44 (29%)</td>
<td></td>
</tr>
<tr>
<td>College graduate or more</td>
<td>112 (67%)</td>
<td>102 (67%)</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>Never married or Partnered</td>
<td>138 (83%)</td>
<td>124 (81%)</td>
<td></td>
</tr>
<tr>
<td>Married or Partnered</td>
<td>25 (15%)</td>
<td>26 (17%)</td>
<td></td>
</tr>
<tr>
<td>Separated or Divorced</td>
<td>3 (2%)</td>
<td>3 (2%)</td>
<td></td>
</tr>
<tr>
<td>Age at menarche (years)*</td>
<td>12.8 ± 1.5</td>
<td>12.7 ± 1.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>154 (93%)</td>
<td>144 (94%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Previously using Contraceptives</td>
<td>84 (51%)</td>
<td>82 (54%)</td>
<td>0.49</td>
</tr>
</tbody>
</table>
**Family History of Breast Cancer**  

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>129 (96%)</td>
<td>114 (97%)</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (4%)</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

**Self-reported Diet (kcal/day)**  

<table>
<thead>
<tr>
<th></th>
<th>1901 ± 420</th>
<th>1933 ± 525</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

**Self-reported Physical Activity (MET-hrs/wk)**  

<table>
<thead>
<tr>
<th></th>
<th>21.9 ± 16.6</th>
<th>21.8 ± 17.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>

**Assessed Fitness (METs at 85% max HR)**  

<table>
<thead>
<tr>
<th></th>
<th>6.9 ± 1.5</th>
<th>7.1 ± 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

Note: For continuous variables, values are mean ± SD, and *P*-values are based on Student’s *t*-tests. For categorical variables, *P*-values are based on chi-square tests.

*a* *n* = 310,  
* b* *n* = 251,  
* c* *n* = 312
<table>
<thead>
<tr>
<th>METs reached at 85% of max HR</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>Change (from Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>7.0 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>0.90 (^a) ± 0.07</td>
</tr>
<tr>
<td>Control</td>
<td>7.0 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>0.12 ± 0.08</td>
</tr>
<tr>
<td>P-value</td>
<td>0.59</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>68.0 ± 0.7</td>
<td>68.0 ± 0.7</td>
<td>-0.03 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>68.8 ± 0.8</td>
<td>69.0 ± 0.8</td>
<td>0.03 ± 0.2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.41</td>
<td>0.37</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>25.0 ± 0.2</td>
<td>25.0 ± 0.2</td>
<td>-0.01 ± 0.06</td>
</tr>
<tr>
<td>Control</td>
<td>25.2 ± 0.2</td>
<td>25.2 ± 0.2</td>
<td>0.01 ± 0.06</td>
</tr>
<tr>
<td>P-value</td>
<td>0.45</td>
<td>0.50</td>
<td>0.85</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>24.6 ± 0.5</td>
<td>24.1 ± 0.5</td>
<td>-0.57 (^a) ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>25.0 ± 0.5</td>
<td>25.0 ± 0.5</td>
<td>-0.04 ± 0.2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.56</td>
<td>0.17</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>Percent Body Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>36.9 ± 0.4</td>
<td>35.9 ± 0.4</td>
<td>-0.95 (^a) ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>37.1 ± 0.4</td>
<td>37.0 ± 0.4</td>
<td>-0.09 ± 0.2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.70</td>
<td>0.06</td>
<td>0.0003</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>39.9 ± 0.4</td>
<td>40.4 ± 0.4</td>
<td>0.55 (^a) ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>40.3 ± 0.4</td>
<td>40.4 ± 0.4</td>
<td>0.07 ± 0.1</td>
</tr>
<tr>
<td>P-value</td>
<td>0.41</td>
<td>0.93</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Self-reported Diet (kcal/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>1898 ± 38</td>
<td>1895 ± 51</td>
<td>-18 ± 51</td>
</tr>
<tr>
<td>Control</td>
<td>1932 ± 40</td>
<td>1711 ± 54</td>
<td>-224 (^a) ± 53</td>
</tr>
<tr>
<td>P-value</td>
<td>0.53</td>
<td><strong>0.011</strong></td>
<td><strong>0.004</strong></td>
</tr>
</tbody>
</table>
Note: Values are means ± SE. Positive values represent increases from baseline while negative values represent decreases from baseline.

*Significant within-group change from baseline ($P < 0.05$).
Table 2.3: Baseline, Follow-up, and Changes from Baseline in Sex Hormone and SHBG Levels

<table>
<thead>
<tr>
<th>Sex Hormone</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estradiol (E2) (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>56 (50-63)</td>
<td>60 (56-64)</td>
<td>2.3 ± 2.8</td>
</tr>
<tr>
<td>Controls</td>
<td>58 (51-65)</td>
<td>62 (58-66)</td>
<td>1.4 ± 3.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.66</td>
<td>0.44</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Bioavailable E2 (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>39 (36-41)</td>
<td>40 (37-43)</td>
<td>1.4 ± 1.9</td>
</tr>
<tr>
<td>Controls</td>
<td>41 (39-44)</td>
<td>43 (40-46)</td>
<td>1.1 ± 2.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.14</td>
<td>0.13</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Free E2 (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>1.3 (1.2-1.4)</td>
<td>1.4 (1.3-1.4)</td>
<td>0.05 ± 0.06</td>
</tr>
<tr>
<td>Controls</td>
<td>1.4 (1.3-1.5)</td>
<td>1.5 (1.4-1.6)</td>
<td>0.04 ± 0.07</td>
</tr>
<tr>
<td>P-value</td>
<td>0.14</td>
<td>0.13</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Estrone sulfate (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>2.0 (1.8-2.2)</td>
<td>2.0 (1.9-2.2)</td>
<td>-0.04 ± 0.06</td>
</tr>
<tr>
<td>Controls</td>
<td>2.2 (2.0-2.4)</td>
<td>2.3 (2.1-2.4)</td>
<td>-0.01 ± 0.06</td>
</tr>
<tr>
<td>P-value</td>
<td><strong>0.040</strong></td>
<td><strong>0.017</strong></td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Testosterone (T) (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>451 (423-482)</td>
<td>446 (419-475)</td>
<td>-8.8 ± 9.0</td>
</tr>
<tr>
<td>Controls</td>
<td>473 (442-506)</td>
<td>464 (435-495)</td>
<td>-13.6 ± 9.5</td>
</tr>
<tr>
<td>P-value</td>
<td>0.32</td>
<td>0.38</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Bioavailable T (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>204 (187-222)</td>
<td>204 (188-222)</td>
<td>-2.7 ± 5.6</td>
</tr>
<tr>
<td>Controls</td>
<td>224 (205-245)</td>
<td>225 (206-246)</td>
<td>-3.0 ± 5.8</td>
</tr>
<tr>
<td>P-value</td>
<td>0.13</td>
<td>0.11</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Free T (pg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>8.8 (7.9-9.7)</td>
<td>8.7 (8.0-9.5)</td>
<td>-0.13 ± 0.25</td>
</tr>
<tr>
<td>Controls</td>
<td>9.2 (8.3-10.2)</td>
<td>9.6 (8.8-10.5)</td>
<td>-0.04 ± 0.30</td>
</tr>
<tr>
<td>P-value</td>
<td>0.50</td>
<td>0.11</td>
<td>0.80</td>
</tr>
</tbody>
</table>
**Progesterone (ng/mL)**

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>12 (10-14)</td>
<td>10 (8-11)</td>
<td>0.61</td>
</tr>
<tr>
<td>Follow-up</td>
<td>13 (11-15)</td>
<td>12 (10-14)</td>
<td>0.12</td>
</tr>
<tr>
<td>Change</td>
<td>0.0 ± 0.9</td>
<td>-1.1 ± 1.0</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**SHBG (nmol/L)**

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>27 (25-30)</td>
<td>26 (24-29)</td>
<td>0.15</td>
</tr>
<tr>
<td>Follow-up</td>
<td>25 (23-27)</td>
<td>24 (22-26)</td>
<td>0.08</td>
</tr>
<tr>
<td>Change</td>
<td>-1.3 ± 1.0</td>
<td>-1.8 ± 1.1</td>
<td>0.74</td>
</tr>
</tbody>
</table>

NOTE: Values are age- and BMI-adjusted geometric means (95% CI) for baseline and follow-up, and mean ± SE for changes in hormone levels. Positive values represent increases from baseline while negative values represent decreases from baseline.

* n = 311, b Significant within-group change from baseline (P < 0.05)
Figure 2.1. CONSORT Diagram Showing Participant Recruitment, Screening, Randomization, and Retention
CHAPTER 3:

URINARY ESTROGEN METABOLITES
I. SUMMARY

**Background:** It is well accepted that exercise can decrease breast cancer risk. Limited clinical evidence suggests that this risk could be mediated through changes in estrogen metabolism in premenopausal women. Our objective was to investigate the effects of exercise on premenopausal estrogen metabolism pertinent to breast cancer risk.

**Methods:** Sedentary, healthy, young eumenorrheic women were randomized into an intervention of 30 minutes of moderate-to-vigorous aerobic exercise 5 times a week for approximately 16 weeks \((n = 212)\), or into a usual-lifestyle sedentary control group \((n = 179)\). Urinary levels of estrogens (estrone \([E_1]\), estradiol \([E_2]\), and estriol) and nine estrogen metabolites were measured at baseline and at study end by liquid chromatography/tandem mass spectrometry. The ratios of 2-hydroxyestrone to 16α-hydroxyestrone \((2-\text{OHE}_1/16\alpha-\text{OHE}_1)\) and 2-\text{OHE}_1 to 4-hydroxyestrone \((2-\text{OHE}_1/4-\text{OHE}_1)\) were also calculated.

**Results:** The exercise intervention resulted in significant increases in aerobic fitness and lean body mass, and a significant decrease in percent body fat. For exercisers who completed the study \((n = 165)\), 2-\text{OHE}_1/16α-\text{OHE}_1 increased significantly \((P = 0.043)\), while \(E_1\) decreased significantly \((P = 0.030)\) in control participants \((n = 153)\). The change from baseline in 2-\text{OHE}_1/16α-\text{OHE}_1 was significantly different between groups \((P = 0.045)\), even after adjustment for baseline values.

**Conclusions:** The exercise intervention resulted in a significant increase in the 2-\text{OHE}_1/16α-\text{OHE}_1 ratio, but no differences in other estrogen metabolites or ratios.
**Impact:** Our results suggest that changes in premenopausal estrogen metabolism may be a mechanism by which increased physical activity lowers breast cancer risk.

**II. INTRODUCTION**

It is well accepted that lifetime estrogen exposure increases the risk for breast cancer as a result of cumulative stimulation of epithelial cell division by estrogen\[297\]. It has also been suggested that some metabolites resulting from the biotransformation and inactivation of estrogen can play a significant role in breast carcinogenesis \[300\].

Specifically, the products resulting from the oxidation of estradiol (E\(_2\)) and estrone (E\(_1\)) known as hydroxyestrogens have been shown to display varying degrees of carcinogenicity. For example, 2-hydroxyestrone (2-OHE\(_1\)) partially antagonizes the growth-stimulatory effect of E\(_2\) in cultured human MCF-7 breast cancer cells [89] while 2-hydroxyestradiol (2-OHE\(_2\)) has little or no carcinogenic activity in Syrian hamsters [301, 302]. In cultured mouse mammary epithelial cells, 16α-hydroxyestrone (16α-OHE\(_1\)) increases unscheduled DNA synthesis and promoted anchorage-independent growth [275, 303]. The metabolite 4-hydroxyestrone (4-OHE\(_1\)) is considered genotoxic due to its redox cycling process, which generates reactive oxygen species (ROS) and highly cytotoxic semiquinone/quinone intermediates that react with DNA [300]. The 2-hydroxyestrogens also undergo redox cycling, but appear to lack carcinogenic activity due to a more rapid clearance *in vivo* [304] associated with a faster rate of inactivation through *O*-methylation [94, 305]. Finally, one product resulting from *O*-methylation,
namely 2-methoxyestradiol (2-MeOE₂), has been shown to be a potent inhibitor of cell proliferation and angiogenesis [96, 306].

Despite evidence suggesting the possible importance of other aspects of estrogen metabolism on breast cancer, human studies have largely focused on the ratio of 2-OHE₁ to 16α-OHE₁ (2-OHE₁/16α-OHE₁). Given the different genotoxic capacity of these metabolites, it has been hypothesized that metabolism favoring the production of 2-OHE₁ over 16α-OHE₁ may be inversely associated with breast cancer risk [307].

In premenopausal women, the strongest evidence in favor of this hypothesis comes from two early prospective studies in which urine specimens were collected several years prior to diagnosis. In the Guernsey III cohort study, women in the highest tertile of urinary 2-OHE₁/16α-OHE₁ ratio had a non-significantly lower odds ratio (0.75) for breast cancer than women in the lowest two tertiles [104]. Similarly, in a study reported by Muti et al., women in the highest quintile of the urinary 2-OHE₁/16α-OHE₁ ratio had an adjusted odds ratio for breast cancer of 0.58, although again this was not statistically significant [105]. In contrast, in a more recent prospective study, a higher 2-OHE₁/16α-OHE₁ ratio was associated with an increase in premenopausal ER-positive breast cancer [102]. However, the association was not statistically significant and estrogen metabolites were measured in serum and not in urine as in the two previous studies.

Significant relationships between premenopausal breast cancer risk and urinary levels of estrogen metabolites and their ratios have been observed in some case-control studies, but findings have been inconsistent. The case-control studies of both Coker et al. [308] and Kabat et al. [309] found an increased risk in women with an increased 2-OHE₁/16α-
OHE\textsubscript{1} ratio, but other studies did not [310-312]. As for other measures, in two other studies, control women had significantly higher levels of 2-hydroxyestrogens, 4-hydroxyestrogens, 16α-OHE\textsubscript{1} [313], and 2-OHE\textsubscript{1}/4-OHE\textsubscript{1} ratio [314] than women with breast cancer.

While the association between estrogen metabolism and breast cancer risk needs further investigation, epidemiological evidence strongly supports the association between higher levels of aerobic exercise and reduced risk for breast cancer [285]. However, whether exercise in premenopausal women results in what may be favorable effects on estrogen metabolism is not clear. For example, in one small study highly fit women exercising strenuously for 368 minutes a week had similar values of 2-OHE\textsubscript{1}, 16α-OHE\textsubscript{1}, and 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} ratio than those of women exercising recreationally for only 60 minutes a week [258]. In contrast, in another small study higher levels of self-reported physical activity were associated with higher urinary concentrations of 2-OHE\textsubscript{1} and a higher 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} ratio [256]. Recently, a large study of 603 women from the NHS-II found high levels of physical activity not only to be correlated with a higher 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} ratio, but also significantly lower levels of E\textsubscript{2} and 16α-OHE\textsubscript{1} [315].

In comparison, data from exercise intervention studies has been conflicting. For instance, in interventions lasting 12 weeks [265], 16 weeks [268], or even 6 months [316], moderate-to-vigorous intensity aerobic exercise in premenopausal women did not result in any significant changes in urinary concentrations of E\textsubscript{1}, E\textsubscript{2}, estriol (E\textsubscript{3}), 2-hydroxyestrogens, 4-hydroxyestrogens, or 16α-OHE\textsubscript{1}, or either 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} or 2-OHE\textsubscript{1}/4-OHE\textsubscript{1} ratios. In two small exercise interventions coupled with calorie restriction
lasting 4 and 6 months, there were significant increases in urinary levels of luteal phase
16α-OHE$_1$ and 2-OHE$_1$/16α-OHE$_1$, respectively [266, 269].

Overall, the data on the effects of aerobic exercise on premenopausal estrogen
metabolism are not only conflicting but also narrow in scope. With the exception of two
studies [268, 315], all published studies to date have focused on a limited number of
estrogen metabolites, namely 2-OHE$_1$ and 16α-OHE$_1$, and their ratio. Furthermore, no
study has yet investigated the levels of the 2- and 4- methylated catecholestrogens despite
their purported role in breast carcinogenesis as suggested by culture and animal studies.

The WISER (Women In Steady Exercise Research) study was a large, randomized,
exercise-controlled, parallel-arm, clinical study investigating the effects of 16-weeks of
moderate-to-vigorous intensity aerobic exercise on several parameters pertinent to breast
cancer risk in sedentary, healthy, young eumenorrheic women. Here we report changes
from baseline in urinary levels of estrogens (E$_1$, E$_2$, and E$_3$), nine estrogen metabolites (2-
OHE$_1$, 2-OHE$_2$, 16α-OHE$_1$, 4-OHE$_1$, 4-hydroxyestradiol [4-OHE$_2$], 2-methoxyestrone,
[2-MeOE$_1$], 2-MeOE$_2$, 4-methoxyestrone [4-MeOE$_1$], and 4-methoxyestradiol [4-
MeOE$_2$]), and two estrogen metabolite ratios (2-OHE$_1$/16α-OHE$_1$, and 2-OHE$_1$/4-OHE$_1$).

III. SUBJECTS AND METHODS

Study design

The WISER study was a randomized clinical trial investigating the effects of a 16-
week aerobic exercise intervention on breast cancer biomarkers of healthy,
premenopausal women. All procedures were approved by the Human Subjects Review
Committee at the University of Minnesota (Institutional Review Board; IRB ID#0505M69867). Written informed consent was obtained from each participant prior to participation. A complete description of the study design, including participant recruitment, screening, randomization, and retention has been published [288].

Briefly, WISER study investigators emailed more than 100,000 female residents of the Minneapolis-St. Paul metropolitan area regarding participation. Women who were interested were screened online based on age (18-30 years old), physical activity (two or less weekly sessions of moderate intensity exercise), smoking status (non-smoking), body mass index (BMI) (18-40 kg/m$^2$ inclusive), and self-reported menstrual cycle length (24 to 35 days). Women who met these criteria were further screened via telephone ($n = 1,684$) and excluded based on previous hormonal contraception use (past three months or 12 months if depot-medroxyprogesterone acetate), gynecological problems, metabolic or endocrine-related diseases, current or recent (past 6 months) pregnancy, non-melanoma cancer in the past 5 years, alcohol consumption (more than 7 servings per week), and body weight changes (more than 10% over the past year).

Of the 966 women who attended a 2-hour orientation, 391 provided written consent and were enrolled in the study. After baseline measurements, women were randomized into either an exercise intervention ($n = 212$) or a no-exercise, usual-lifestyle control group ($n = 179$) for approximately 16 weeks. Randomization was stratified on baseline BMI tertiles ($\leq 22.8$, $22.8-26.3$, $\geq 26.3$) based on the 50$^{th}$ and 75$^{th}$ percentiles from NHANES I data and age (18-24 vs. 25-30). Participants who failed to return for follow-up measures were dropped from of the study. Additionally, exercisers were subject to
study exclusion if they missed 15 or more exercise sessions. Figure 3.1 shows the recruitment, screening, randomization, retention, and completion of WISER participants.

**Exercise intervention**

Women randomized to the exercise intervention trained aerobically five times a week for 30 minutes on a treadmill, stair-stepper, or elliptical machine, at a specified intensity based on age-predicted maximal heart rate (max HR) for 16 weeks (± 2 weeks). The exercise intensity was initially set at 65%-70% of the age-predicted max HR and was gradually increased by 5% every four weeks until 80%-85% of age-predicted max HR was reached (stage 1 = 65%-70%; stage 2 = 70%-75%; stage 3 = 75%-80%; stage 4 = 80%-85%).

All training sessions took place at the University of Minnesota’s Recreation Center. At the first training session, a certified personal trainer provided instruction on the proper use of the exercise machines, heart rate monitor and watch, and completion of an exercise log after each workout. Trainers supervised exercise sessions and reviewed the exercise logs at least once weekly to monitor adherence and safety. When not meeting with a trainer, participants were expected to complete the remaining of the workout sessions unsupervised. Exercise adherence was assessed using the data from the heart rate monitor (Polar Electro Inc., Lake Success, NY) and exercise logs.

Any physical activity performed after randomization and outside the prescribed exercise intervention was assessed at the end of the study with a physical activity questionnaire administered by a research staff member. All participants, regardless of randomization outcome, were asked to maintain their baseline body weight. Control
participants were asked to not only to maintain their usual level of physical activity but also to not change their eating habits.

**Anthropometrics**

Body mass was measured to the nearest 0.1 kg using an electronic scale (Scale Tronix, White Plains, NY) 4 times throughout the study (baseline, intervention weeks 4 and 8, and follow-up). Height was measured without shoes to the nearest 0.1 cm (Scale Tronix, White Plains, NY) by a stadiometer at baseline. Body mass index was calculated by dividing body mass in kg by height in meters squared (kg/m$^2$). Body composition was assessed at baseline and follow-up by dual energy x-ray absorptiometry (DXA) using a Lunar Prodigy DXA apparatus (Lunar Radiation Corp., Madison, WI).

**Aerobic fitness and physical activity**

Aerobic fitness was assessed at baseline and immediately after the intervention with a sub-maximal treadmill test described previously [288]. This workload was then converted to metabolic equivalents (METs) by using a standard conversion formula [289]. Self-reported physical activity performed a year prior to the study and during the 4-month follow-up period was assessed by a research staff using a modified version of the Modifiable Activity Questionnaire [290]. This information was transformed into MET-hours per week (MET-hrs/wk) using commonly accepted MET values [291].

**Dietary intake**

Usual dietary intake was assessed through self-reported, 3-day food records completed concomitantly with the urine collections at baseline and follow-up. Nutrient intake was determined using The Food Processor SQL® by ESHA Research (Salem, OR).
**Urine collection**

Forty-eight hours prior to the urine collection, participants were asked to avoid moderate or vigorous exercise and abstain from alcohol. Urine was collected for three consecutive 24-hour periods in the mid-follicular phase (follicular days 7 - 9 of baseline menstrual cycle 2 and follow-up menstrual cycle 6). Throughout each day, urine was collected in a 1-liter bottle and kept cold with ice packs inside an insulated bag. At the end of each collection day, urine was transferred into a 3-liter bottle containing ascorbic acid (1 mg/mL) to prevent oxidation, and stored in a home refrigerator or cooler provided by the study. Once the urine collection was completed, collection bottles were retrieved by a research staff member and brought to the General Clinical Research Center at the University of Minnesota for processing. Urine was refrigerated and 0.1% sodium azide was added before the three 24-hour collections were pooled. Aliquots were taken and stored at -20 °C until analysis.

**Estrogen metabolites**

Urinary estrogens (E₁, E₂, and E₃) and their metabolites (2-OHE₁, 2-OHE₂, 16α-OHE₁, 4-OHE₁, 4-OHE₂, 2-MeOE₁, 2-MeOE₂, 4-MeOE₁, and 4-MeOE₂) were analyzed in the mid-follicular phase of baseline and follow-up cycles by liquid chromatography/tandem mass spectrometry (LC/MS-MS) performed using a Thermo Electron Quantum Discovery Max Triple Quadrupole LC-MS/MS instrument [106]. Quantitative analysis was performed using Thermo Electron Xcalibur proprietary software.
Samples with non-detectable levels were assigned values of the lowest detectable standard (0.014 ng/mL urine). Concentrations were expressed both as nanomol per day (nmol/day) and nanograms per milligram of creatinine (ng/mg Cr). Urinary creatinine was analyzed at the Fairview University Diagnostic Laboratories.

Samples were run in duplicate and in batches such that each batch contained both baseline and follow-up samples from each participant and an equal number of exercise and control participants. One quality control sample was included in each batch. The mean intra-assay and inter-assay CVs were 5.1% and 13.4% for E1; 5.2% and 16.0% for E2; 5.6% and 11.4% for E3; 4.2% and 12.3% for 2-OHE1; 7.7% and 10.8% for 2-OHE2; 6.2% and 18.7% for 16α-OHE1; 4.3% and 12.2% for 4-OHE1; 14.0% and 51.2% for 4-OHE2; 7.0% and 11.3% for 2-MeOE1; 5.8% and 10.0% for 2-MeOE2; 7.4% and 10.3% for 4-MeOE1; and 6.9% and 7.9% for 4-MeOE2.

Statistical analyses

Unadjusted comparisons of baseline characteristics were performed using Student’s t-tests for continuous variables and chi-square tests for categorical variables.

Two estrogen metabolite ratios of interest, namely the 2-OHE1/16α-OHE1 ratio and 2-OHE1/4-OHE1 ratio, were calculated by dividing the concentration of 2-OHE1 by either 16α-OHE1 or 4-OHE1, respectively.

Baseline associations between urinary estrogens, their metabolites, and metabolite ratios and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were determined using Spearman correlation coefficients.
The main study analysis assessed the intervention effects using only data from participants who completed the baseline and follow-up urine collection regardless of compliance level. Baseline and follow-up comparisons were conducted using log-transformed values and results are presented as geometric means with 95% confidence intervals. Changes from baseline comparisons were compared on the original scale. All comparisons were adjusted for study-design age and BMI strata with a general linear model. When there were significant differences at baseline in an outcome, follow-up and change from baseline comparisons were additionally adjusted for baseline values. Linear models were calculated using SAS software, version 9.2 (SAS institute Inc., 2008, Cary, NC). \( P < 0.05 \) was considered statistically significant. Estrogen metabolite data were analyzed as both nmol/day and ng/mg Cr. Results from the two analyses did not differ significantly, and we report results as nmol/day.

**IV. RESULTS**

*Study participants*

Of the 212 and 179 women randomized into the exercise and control groups, 165 (77.8%) and 153 (85.5%), respectively, completed the WISER study. With the exception of education (47% of drop-outs had some college education vs. 27% of study completers \( P = 0.002 \)), drop-outs were no different from women who completed the study in any of the baseline demographic characteristics measured (data not shown). Also, there were no significant differences between exercisers and controls in baseline demographic characteristics (Table 3.1). In general, women who completed the study were mostly
Caucasian (72%), single (82%), nulliparous (93%), had education beyond high school (96%), and had no first-degree relatives with breast cancer (97%).

**Baseline estrogen metabolism**

With the exception of 2-OHE\textsubscript{1} ($P = 0.084$) and 2-OHE\textsubscript{1}/16α-OHE\textsubscript{1} ratio ($P = 0.044$), exercisers had similar levels of urinary estrogens, estrogen metabolites, and 2-OHE\textsubscript{1}/4-OHE\textsubscript{1} ratio than control participants at baseline (Table 3.2). No significant baseline associations between any of the urinary endpoints and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were found.

Overall, the concentration of estrone and its metabolites were higher than their estradiol counterparts, especially for E\textsubscript{1}, 2-OHE\textsubscript{1}, 4-OHE\textsubscript{1}, and 4-MeOE\textsubscript{1} as compared to E\textsubscript{2}, 2-OHE\textsubscript{2}, 4-OHE\textsubscript{2}, and 4-MeOE\textsubscript{2}, respectively. Estrogen hydroxylation showed an isomeric preference for the C-2 position. Specifically, concentrations of 2-OHE\textsubscript{1} were about 14- and 20-fold higher than those of 16α-OHE\textsubscript{1} and 4-OHE\textsubscript{1}, respectively, and concentration of 2-OHE\textsubscript{2} about 40-fold those of 4-OHE\textsubscript{2}.

**Exercise adherence**

On average, exercise participants completed 127 minutes per week of the assigned 150 minutes of exercise intervention. Details about exercise adherence and compliance can be found elsewhere [317].

**Intervention effects**

The exercise intervention resulted in significant improvements in body composition and aerobic fitness without changes in body weight. As previously reported, exercisers experienced significant increases in aerobic fitness (0.90 METs reached at 85% of max...
HR vs. 0.12 METs in controls) and lean body mass (0.55kg vs. 0.07 kg), as well as significant decreases in fat mass (0.57 kg vs. 0.04 kg) and percent body fat (0.95% vs. 0.09%). In contrast, control participants experienced no changes in body composition, aerobic fitness, and body weight despite a significant reduction in daily caloric intake (-224 kcal/day). Exercisers also reduced their food consumption, but only by 18 kcal/day ($P > 0.05$). Details on the effects of this intervention on body composition, body weight, aerobic fitness, and energy intake have been published previously [318].

As previously reported, the exercise intervention resulted in no significant changes in endogenous levels of E$_2$, estrone sulfate, progesterone, T, or SHBG (38). Exercisers, however, did experience a significant increase in urinary 2-OHE$_1$/16α-OHE$_1$ ratio ($P = 0.043$) while controls had a non-significant decrease in 2-OHE$_1$/16α-OHE$_1$ ratio. The difference in the change from baseline in 2-OHE$_1$/16α-OHE$_1$ ratio between groups was significant ($P = 0.045$), even after adjustment for baseline values. Figure 3.2 shows that many, but not all, of the participants who experienced an absolute change in 2-OHE$_1$/16α-OHE$_1$ ratio greater than 100 had a high baseline 2-OHE$_1$/16α-OHE$_1$ ratio. Levels of E$_1$ remained unchanged in exercisers but decreased in controls resulting in a statistically significant change from baseline between the groups ($P = 0.042$). No significant within-group changes or between-group differences at follow-up were observed for other estrogens, estrogen metabolites, or ratios.
V. DISCUSSION

We found that in healthy premenopausal women, an exercise regimen of 150 minutes of moderate-to-vigorous aerobic exercise per week for 16 weeks resulted in significant changes in estrogen metabolism in a direction consistent with reduction of breast cancer risk. Specifically, exercise participants experienced a significant increase in urinary levels of 2-OHE$_1$ and a small non-significant decrease in 16α-OHE$_1$ levels. These changes resulted in a significant increase in the 2-OHE$_1$/16α-OHE$_1$ ratio. In contrast, women in the control group had a non-significant decrease in 2-OHE$_1$/16α-OHE$_1$ ratio largely attributable to those few controls with large baseline ratio having large decreases in the ratio. Controls also had an unexplained significant decrease in E$_1$. We did not find evidence for exercise resulting in changes in the 4-hydroxylation pathway or other differences that conceivably could have been found.

Overall, our results differ from those of other exercise intervention studies investigating the effects of aerobic exercise on premenopausal estrogen metabolism. For instance, in a small 5-month weight-loss clinical trial involving moderate-intensity exercise, both controls and exercisers had significant increases in the urinary 2-OHE$_1$/16α-OHE$_1$ ratio, but in contrast to our results, the change in ratio between the groups was not statistically significant [266]. In a small pre-post design study, 4 months of moderate exercise coupled with calorie restriction resulted in non-significantly higher urinary levels of 2-OHE$_1$ and 16α-OHE$_1$ and the ratio [269]. In our study, both 2-OHE$_1$ and the 2-OHE$_1$/16α-OHE$_1$ ratio increased significantly, whereas 16α-OHE$_1$ decreased non-significantly. Both of these studies differed from our study in that aerobic exercise
was coupled with significant calorie restriction making it impossible to discern whether
the changes reported were the result of the exercise or diet.

When compared to exercise-only interventions, our study remains the only one to
report significant changes in estrogen metabolism. For example, in a moderate-to-
vigorous aerobic exercise intervention lasting 12 weeks (30-45 minutes, 4 days per
week), Campbell and colleagues reported no significant changes in urinary
premenopausal levels of 2-OHE$_1$, 16$\alpha$-OHE$_1$, or 2-OHE$_1$/16$\alpha$-OHE$_1$ ratio [265].
Similarly, in a pilot study carried out by our research group, total levels of 2-OHE (2-
OHE$_1$ + 2-OHE$_2$), 4-OHE (4-OHE$_1$ + 4-OHE$_2$), and the 2-OHE$_1$/16$\alpha$-OHE$_1$ ratio
remained unchanged in 15 young women exercising aerobically for 30 minutes a day, 5
times a week for 16 weeks [268]. Similarly, Robles-Gil et al. did not find significant
changes in E$_1$, E$_2$, or E$_3$ in 20 premenopausal women after 6 months of 60 minutes of
moderate-intensity exercise 3 days/week [316].

A possible explanation for the disparity between the results reported by these exercise
intervention studies and our study may be found in the choice of study design and
methodology. The WISER study had many methodological advantages over previously
published research. First, the sample size in our study ($n = 318$) was an order of
magnitude or more larger than those of the other three studies. Second, our study design
utilized randomized controls (only Campbell et al. study was randomized). Third, unlike
the studies of Campbell et al. and Robles-Gil et al., in which first morning urine samples
were used, WISER participants collected three 24-hour urine collections allowing for a
more robust and representative analysis of the chronic effect of aerobic exercise on
estrogen metabolism. Finally, our study provides the most comprehensive analysis on the effects of exercise on estrogen metabolism to date. We have analyzed urinary levels of the major parent estrogens (E₁, E₂ and E₃) and nine of their estrogen metabolites by LC/MS-MS. This newer methodology is considered to be superior not only to the ELISA methods employed by these studies but also to the current gold standard GC-MS due to its increased sensitivity and sample throughput [106, 319]. Unlike Xu and colleagues, we were able to quantify and report 4-OHE₂ concentrations, although its lack of detection in 53.4% of our samples resulted in a higher-than-expected inter-assay CV.

Altogether, the findings of the WISER study are significant because they provide the first clinical evidence that aerobic exercise can significantly change estrogen metabolism in premenopausal women. Specifically, our results show that such an exercise intervention can lead to increases in 2-OHE₁ while having little effect in 16α-OHE₁, which ultimately result in significant increases in the 2-OHE₁/16α-OHE₁ ratio. Importantly, increases in this ratio have been associated with a significant reduction in breast cancer risk.

From a clinical point of view, the assessment of urinary 2-OHE₁/16α-OHE₁ ratio is also relevant as it has been found to be a good approximation to the 2-OHE₁/16α-OHE₁ ratio of breast tissue [320]. Perhaps one mechanism by which exercise mediates estrogen metabolism is through the regulation of P-450 cytochrome enzymes responsible for controlling estrogen hydroxylation and catecholestrogen methylation. Given the implication these results have for breast cancer prevention efforts, future studies should
not only attempt to corroborate our results, but also investigate the exact mechanisms by which exercise leads to these favorable estrogen metabolism changes.
Table 3.1: Baseline Characteristics of Randomized WISER Participants ($n = 318$)

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Exercisers ($n = 165$)</th>
<th>Controls ($n = 153$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SE)</td>
<td>25.4 ± 0.3</td>
<td>25.2 ± 0.3</td>
</tr>
<tr>
<td>Not Married or Partnered ($n$, %)</td>
<td>137 (83%)</td>
<td>124 (81%)</td>
</tr>
<tr>
<td>Education beyond High School ($n$, %)</td>
<td>157 (95.2%)</td>
<td>148 (96.7%)</td>
</tr>
<tr>
<td>Caucasian ($n$, %)</td>
<td>123 (75%)</td>
<td>107 (70%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body Composition (mean ± SE)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>67.5 ± 1.1</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.8 ± 0.4</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>36.4 ± 0.7</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>24.3 ± 0.9</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>39.8 ± 0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reproductive Characteristics</th>
<th>Exercisers ($n = 165$)</th>
<th>Controls ($n = 153$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Menarche, years (mean ± SE) (a)</td>
<td>12.7 ± 0.12</td>
<td>12.7 ± 0.11</td>
</tr>
<tr>
<td>Nulliparous ($n$, %)</td>
<td>153 (93%)</td>
<td>144 (94%)</td>
</tr>
<tr>
<td>Previous Contraceptive Use ($n$, %)</td>
<td>84 (51%)</td>
<td>82 (54%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family History of Breast Cancer (b)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No ($n$, %)</td>
<td>129 (96%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical Activity, Fitness &amp; Diet (mean ± SE)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate Exercise (MET-hrs/wk)</td>
<td>1902 ± 421</td>
</tr>
<tr>
<td>Aerobic Fitness (METs at 85% max HR)</td>
<td>21.9 ± 1.3</td>
</tr>
<tr>
<td>Total calorie intake (kcal/day) (c)</td>
<td>6.9 ± 0.1</td>
</tr>
</tbody>
</table>

Note: There were no significant differences at baseline between study groups for any of these variables

\(a n = 310, b n = 251, c n = 312\)
<table>
<thead>
<tr>
<th></th>
<th>Baseline Geometric Mean (95% CI)</th>
<th>P-value for Baseline Differences</th>
<th>Follow Up Geometric Mean (95% CI)</th>
<th>P-value for Changes in Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estrone, E₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>23.1 (19.0 – 28.2)</td>
<td>0.586</td>
<td>23.0 (18.9 – 28.0)</td>
<td>0.042</td>
</tr>
<tr>
<td>Controls</td>
<td>25.0 (20.3 – 30.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Estradiol, E₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>7.8 (6.8 – 8.8)</td>
<td>0.786</td>
<td>8.3 (7.2 – 9.5)</td>
<td>0.725</td>
</tr>
<tr>
<td>Controls</td>
<td>8.0 (7.0 – 9.1)</td>
<td></td>
<td>8.0 (7.2 – 9.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Estriol, E₃</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>21.3 (16.5 – 27.4)</td>
<td>0.963</td>
<td>18.6 (14.4 – 24.2)</td>
<td>0.715</td>
</tr>
<tr>
<td>Controls</td>
<td>21.1 (16.2 – 27.5)</td>
<td></td>
<td>21.0 (16.0 – 27.5)</td>
<td></td>
</tr>
<tr>
<td><strong>16α-OHE₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>2.9 (2.3 – 3.7)</td>
<td>0.303</td>
<td>2.6 (2.1 – 3.4)</td>
<td>0.971</td>
</tr>
<tr>
<td>Controls</td>
<td>2.5 (1.9 – 3.1)</td>
<td></td>
<td>2.5 (1.9 – 3.2)</td>
<td></td>
</tr>
<tr>
<td><strong>2-OHE₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>39.3 (33.7 – 45.8)</td>
<td>0.084</td>
<td>44.2 (38.4 – 50.8)</td>
<td>0.098</td>
</tr>
<tr>
<td>Controls</td>
<td>47.6 (40.5 – 55.8)</td>
<td></td>
<td>45.5 (39.3 – 52.6)</td>
<td></td>
</tr>
<tr>
<td><strong>4-OHE₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>2.3 (2.1 – 2.7)</td>
<td>0.647</td>
<td>2.4 (2.1 – 2.7)</td>
<td>0.362</td>
</tr>
<tr>
<td>Controls</td>
<td>2.4 (2.1 – 2.8)</td>
<td></td>
<td>2.4 (2.1 – 2.7)</td>
<td></td>
</tr>
<tr>
<td><strong>2-OHE₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>3.4 (2.4 – 5.0)</td>
<td>0.209</td>
<td>3.5 (2.4 – 5.1)</td>
<td>0.194</td>
</tr>
<tr>
<td>Controls</td>
<td>2.5 (1.7 – 3.6)</td>
<td></td>
<td>2.9 (1.9 – 4.2)</td>
<td></td>
</tr>
<tr>
<td><strong>4-OHE₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>0.7 (0.4 – 1.0)</td>
<td>0.767</td>
<td>0.8 (0.5 – 1.3)</td>
<td>0.898</td>
</tr>
<tr>
<td>Controls</td>
<td>0.7 (0.5 – 1.2)</td>
<td></td>
<td>0.5 (0.3 – 0.8)</td>
<td></td>
</tr>
<tr>
<td><strong>2-MeOE₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>8.2 (6.5 – 10.4)</td>
<td>0.312</td>
<td>9.3 (7.4 – 11.8)</td>
<td>0.406</td>
</tr>
<tr>
<td>Controls</td>
<td>6.9 (5.4 – 8.8)</td>
<td></td>
<td>8.6 (6.8 – 10.9)</td>
<td></td>
</tr>
</tbody>
</table>
### 4-MeOE<sub>1</sub>

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>T-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 (1.4 – 2.6)</td>
<td>2.2 (1.6 – 3.0)</td>
<td>0.562</td>
<td>1.7 (1.2 – 2.4)</td>
<td>1.5 (1.1 – 2.1)</td>
</tr>
</tbody>
</table>

### 2-MeOE<sub>2</sub>

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>T-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 (5.4 – 9.3)</td>
<td>5.8 (4.3 – 7.6)</td>
<td>0.269</td>
<td>7.0 (5.4 – 9.1)</td>
<td>6.6 (5.1 – 8.7)</td>
</tr>
</tbody>
</table>

### 4-MeOE<sub>2</sub>

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>T-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 (1.2 – 2.3)</td>
<td>1.2 (0.9 – 1.8)</td>
<td>0.254</td>
<td>1.5 (1.0 – 2.1)</td>
<td>0.9 (0.7 – 1.4)</td>
</tr>
</tbody>
</table>

### 2-OHE<sub>1</sub>/16α-OHE<sub>1</sub>

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>T-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.4 (10.4 – 17.2)</td>
<td>19.3 (14.8 – 25.1)</td>
<td>0.044</td>
<td>16.8 (13.1 – 21.4)</td>
<td>18.5 (14.3 – 24.0)</td>
</tr>
</tbody>
</table>

### 2-OHE<sub>1</sub>/4-OHE<sub>1</sub>

<table>
<thead>
<tr>
<th></th>
<th>Exercisers</th>
<th>Controls</th>
<th>T-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.8 (14.8 – 19.0)</td>
<td>19.5 (17.1 – 22.2)</td>
<td>0.104</td>
<td>18.7 (16.8 – 20.9)</td>
<td>18.9 (16.9 – 21.2)</td>
</tr>
</tbody>
</table>

**NOTE:** Values are age- and BMI-adjusted geometric means (95% CI). Follow-up and mean change from baseline comparisons in 2-OHE<sub>1</sub> and 2-OHE<sub>1</sub>/16α-OHE<sub>1</sub> were additionally adjusted for baseline values.

Within-group differences: *P*-value = 0.030, ^P*-value = 0.043
Figure 3.1 CONSORT Diagram Showing Participant Recruitment, Screening, Randomization, and Retention.
Figure 3.2. Changes in Baseline vs. Baseline in 2-OHE₁/16α-OHE₁ Ratio
CHAPTER 4:

DISSERTATION SUMMARY
The purpose of this dissertation was to investigate the effects of a 16-week aerobic exercise intervention on hormonal breast cancer biomarkers such as endogenous sex hormones and urinary estrogen metabolites in premenopausal women. Moderate-to-vigorous aerobic exercise performed for 30 minutes, five times a week resulted in significant improvements in aerobic fitness and body composition measures. While no body weight changes were observed in either study group, women in the exercise group experienced significant increases in lean body mass and decreases in percent body fat.

With the exception of progesterone, endogenous sex hormones and SHBG levels did not change in either group. In exercisers, progesterone levels decreased significantly, albeit this change was similar to that of the control group suggesting a null effect by the exercise intervention.

Similarly, no changes in urinary estrogen and estrogen metabolite levels were observed with the exception of a borderline statistically significant increase of 12.5% in 2-OHE$_1$ levels and a significant increase of 25% in the 2-OHE$_1$/16α-OHE$_1$ ratio. Specifically, in exercisers the 2-OHE$_1$/16α-OHE$_1$ ratio increased significantly when compared to baseline levels ($P = 0.043$) and also when compared to the control group ($P = 0.045$), even after the adjustment for baseline 2-OHE$_1$/16α-OHE$_1$ ratio.

A closer look into this data revealed those participants with a higher baseline 2-OHE$_1$/16α-OHE$_1$ ratio experienced the greatest changes in 2-OHE$_1$/16α-OHE$_1$ ratio due to the intervention. This observation can be of particular importance for future exercise intervention studies, which will seem to benefit the most by recruiting female participants based on baseline 2-OHE$_1$/16α-OHE$_1$ ratio. Similarly, prospective cohort and nested
case-control studies could benefit by recruiting disease-free women with higher 2-OHE$_{1}$/16α-OHE$_{1}$ ratio to assess the impact physical activity levels has on the incidence of breast cancer in this population.

To date, only one other exercise intervention has determined whether a woman’s baseline 2-OHE$_{1}$/16α-OHE$_{1}$ ratio was predictive of her change in 2-OHE$_{1}$/16α-OHE$_{1}$ ratio due to the intervention. The uncontrolled study of Westerlind and Williams, tested an exercise intervention targeted to increase caloric expenditure by 20% coupled with a daily caloric reduction of 20-35% for 4 menstrual cycles in 24 healthy, sedentary, eumenorrheic, premenopausal women [269]. Daily urine spot samples were collected everyday during the baseline cycle and the subsequent four intervention cycles. Urinary concentrations of 2-OHE$_{1}$ and 16α-OHE$_{1}$ were measured via ELISA methods and the 2-OHE$_{1}$/16α-OHE$_{1}$ ratio was calculated manually.

In this study, luteal levels of 2-OHE$_{1}$ marginally decreased ($P = 0.06$) while luteal levels of 16α-OHE$_{1}$ decreased significantly ($P = 0.02$). The luteal 2-OHE$_{1}$/16α-OHE$_{1}$ ratio, however, did not significantly changed from baseline. Similarly, no significant changes were observed in the follicular levels of 2-OHE$_{1}$, 16α-OHE$_{1}$, or 2-OHE$_{1}$/16α-OHE$_{1}$ ratio. As per the design of the study, women on average increased their VO$_{2}$ max by 26% while reducing their daily caloric intake by 421 kcal. The increased energy deficit coupled with reduced caloric intake resulted significant losses in body weight (3.8 kg) and body fat (4.6 %) but no significant changes in lean body mass.

Interestingly, an inverse association was found between a woman’s baseline 2-OHE$_{1}$/16α-OHE$_{1}$ ratio and her overall percent change in 2-OHE$_{1}$/16α-OHE$_{1}$ ratio due to
the intervention ($r = -0.68$, $P < 0.001$). Women divided into tertiles by baseline 2-OHE$_1$/16α-OHE$_1$ ratio were found to be statistically similar in terms of baseline body composition measures, aerobic fitness, daily caloric intake, energy deficit, and menstrual cycle length. However, women in the lowest tertile experienced a significantly greater percent and absolute change in the 2-OHE$_1$/16α-OHE$_1$ ratio in both the follicular and luteal phase than women in the other two tertiles. Based on these results, the authors concluded women with higher 2-OHE$_1$/16α-OHE$_1$ ratio may not be responsive to exercise/caloric-restriction interventions and speculate these women would not benefit from such interventions as they are already at lower risk of developing breast cancer because of their preexisting high 2-OHE$_1$/16α-OHE$_1$ ratio.

The results from this study are in direct contradiction to the findings of the WISER study in which women with the highest, not lowest, 2-OHE$_1$/16α-OHE$_1$ ratio experienced the greatest increases in 2-OHE$_1$/16α-OHE$_1$ ratio due to the intervention. It is important to note that Westerlind and Williams’s study had significant limitations including a small sample size ($n = 24$) and a lack of a control group. In comparison, the WISER study randomized 212 women into the exercise group and 179 into a control group. Furthermore, the WISER study employed a more accurate, sensitive, and specific lab methodology (i.e., LC-MS/MS) than the ELISA method used in this study. Lastly, it is impossible to determine whether the changes reported in this study were a function of the exercise, calorie restriction, weight loss, or a combination of these factors whereas in the WISER study, the changes observed in 2-OHE$_1$/16α-OHE$_1$ ratio can only be attributed to the exercise intervention as diet and weight loss were carefully controlled. Clearly, the
possibility that baseline 2-OHE\(_1\)/16\(\alpha\)-OHE\(_1\) ratio can predict the extent of change in 2-OHE\(_1\)/16\(\alpha\)-OHE\(_1\) ratio needs to be addressed by future exercise interventions.

Collectively, these findings suggest the positive effects aerobic exercise allegedly has on premenopausal breast cancer risk may be mediated through hormonal mechanisms that involve changes in estrogen metabolism but not necessarily changes in endogenous sex hormone levels. Furthermore, these results support the current public health recommendations that promote at least 150 minutes of moderate-intensity aerobic exercise per week in adults not only to maintain health and prevent chronic disease, but also to help prevent breast cancer as well.


APPENDIX A-1

RADIOIMMUNOASSAY METHODS
ESTRADIOL RIA
DSL-4400

Estradiol RIA
Date of assay: ____________
Time samples thawed: __________
Time RIA kit to room temp: _______ (label and leave precipitating reagent in refrigerator)

Allow all reagents to reach room temperature and mix liquid reagents thoroughly by gentle inversion before use.

Label and arrange 12x75mm plastic tubes in triplicate for Total Counts, Non-Specific Binding (NSB), Standards, Controls, and Unknowns (samples).

Load assay tubes. Time: _________

**Do not add anything to TC tubes 1, 2**

Pipette 200 µL of Standard A (0 pg/mL) into NSB tubes 3, 4

Pipette 100 µL of Standard A into tubes 5, 6
  " Standard B into tubes 7, 8
  " Standard C into tubes 9, 10
  " Standard D into tubes 11, 12
  " Standard E into tubes 13, 14
  " Standard F into tubes 15, 16
  " Standard G into tubes 17, 18

Pipette 100 µL of Level I control to tubes 19, 20
  " Level II control to tubes 21, 22
  WISER Control I to tubes 23, 24
  WISER Control II to tubes 25, 26

Pipette 100 µL Unknown samples (in duplicate) to tubes 27+

Mix two Estradiol [I-125] Reagent bottles. Time: _________

Immediately add 100 µL of the Estradiol [I-125] Reagent to all tubes. Time: _________
Use 2.5mL pipette tip, position 2 with repeating pipette

Add 100 µL of the Estradiol Antiserum to all tubes except NSB and TC tubes (1-4). Time: _________
Use 2.5mL pipette tip, position 2 with repeating pipette

Vortex tubes gently (level 4, ~30 sec): Time: _________
Cover with paraffin and incubate **all test tubes** at 37 °C for 30 minutes in the water bath. Time: ________

Allow Precipitating Reagent to reach room temp. Time: ________ *Shake reagent thoroughly*

Add 1.0 mL of Precipitating Reagent (shake thoroughly) to all tubes except TC. Time: ________
Use 12.5 mL pipette tip, position 4 with repeating pipette

Cover test tube rack with paraffin and immediately vortex all tubes.

Incubate at room temperature for 15-20 minutes. Time: ________

Centrifuge all tubes except TC 15-20 minutes at 3000 rpm

Decant all tubes except TC:
- Placing all tubes into a sponge rack
- Inverting sponge rack into radioactive waste container
- Allowing tubes to drain on chux for 1-2 minutes
- Gently blotting droplets on rims
- Returning to upright position
- Allowing to dry upright for 1-2 hours

Return TC tubes back into the test tube rack.

Count **all** tubes in the Gamma counter (1 min):
- Call lab 4-5348
- Take floppy diskette
- Sign-in sheet date, your initials, Kurzer, # tubes
- Insert diskette
- Drawer under the gamma counter contains protocol clips
- Place # 5 clip on left side of 1st tray (1st tray is furthest from you)
- Load tubes in trays from right to left
- Do not load any tubes in stop rack
- Place the stop rack behind all of your trays
- Make sure trays are perpendicular and notch is in the tray groove
- Use function keys to run protocol
- Remove clip from tray and place in numerical order in drawer
- Eject diskette
- Get disk, printout, and make sure area is left clean and organized
- If anything goes awry contact Alma (lab) 5-8292 or (cell) 909-964-7283 and Wanda (o) 5-1249, (barn) 4-3062
ESTRONE SULFATE RIA  
DSL-5400

**Estrone RIA**

Date of assay: __________

Time samples thawed: __________

Time RIA kit to room temp: _______ (leave precipitating reagent in refrigerator)

Allow all reagents to reach room temperature and mix liquid reagents thoroughly by gentle inversion before use.

Label and arrange 12x75 mm **plastic tubes** in duplicate for Total Counts, Non-Specific Binding (NSB), Standards, Controls, and Unknowns (samples).

Load assay tubes. Time: __________

**Do not add anything to TC tubes 1, 2**

Pipette 200 □L of Standard A (0 ng/mL) into **NSB** tubes 3, 4

Pipette 100 □L of Standard A into tubes 5, 6

" Standard B into tubes 7, 8

" Standard C into tubes 9, 10

" Standard D into tubes 11, 12

" Standard E into tubes 13, 14

" Standard F into tubes 15, 16

" Standard G into tubes 17, 18

Pipette 100 □L of Level I control to tubes 19, 20

" Level II control to tubes 21, 22

WISER Control I to tubes 23, 24

WISER Control II to tubes 25, 26

Pipette 100 □L Unknown samples (in duplicate) to tubes 27+

Add 500 □L of the Estrone Sulfate [I-125] Reagent to all tubes. Time: __________

Use a repeating pipette in position 2 with a 12.5 mL pipette tip.

Add 100 □L of the Estrone Sulfate Antiserum to all tubes except **NSB** and TC tubes (1-4).

Time: __________

Use a repeating pipette in position 2 with a 2.5mL pipette tip.

Vortex tubes gently for 1-2 seconds: Time: __________
Cover all test tubes with parafilm and incubate at room temperature for 3 hours on a shaker at 180 rpm. Time: _________

Allow Precipitating Reagent to reach room temp. Time: _________ Shake reagent thoroughly

Add 1.0 mL of Precipitating Reagent (shake thoroughly) to all tubes except TC. Time: _________

Use a repeating pipette in position 4 with a 12.5 mL pipette tip.

Cover test tube rack with paraffin and immediately vortex all tubes.

Incubate all tubes at room temperature for 15 minutes. Time: _________

Centrifuge all tubes except TC for 15 minutes at 3000 rpm.

Decant all tubes except TC by:
   Placing all tubes into a sponge rack
   Inverting sponge rack into radioactive waste container
   Allowing tubes to drain on chux for 1-2 minutes
   Gently blotting droplets on rims
   Returning to upright position
   Allowing to dry upright for 1-2 hours

Return TC tubes back into the test tube rack.

Count all tubes in the Gamma counter (1 min):
   • Call lab 4-5348
   • Take floppy diskette
   • Sign-in sheet date, your initials, Kurzer, # tubes
   • Insert diskette
   • Drawer under the gamma counter contains protocol clips
   • Place # 20 clip on left side of 1st tray (1st tray is furthest from you)
   • Load tubes in trays from right to left
   • Do not load any tubes in stop rack
   • Place the stop rack behind all of your trays
   • Make sure trays are perpendicular and notch is in the tray groove
   • Use function keys to run protocol
   • Remove clip from tray and place in numerical order in drawer
   • Eject diskette
   • Get disk, printout, and make sure area is left clean and organized
   • If anything goes awry contact Alma (lab) 5-8292 or (cell) 909-964-7283 and Wanda (o) 5-1249, (barn) 4-3062
PROGESTERONE
DSL-3900

Progesterone RIA
Date of assay: ____________
Time samples thawed: ______________
Time IRMA kit to room temp: ____________

Allow all reagents to reach room temperature and mix liquid reagents thoroughly by gentle inversion before use.

Label and arrange tubes in duplicate for Total Counts (non-coated plastic test tubes), Standards (coated tube), Controls (coated tube) and Unknowns (coated tube).

Reconstitute Standards and Controls using ddH20.
Use same pipette tip to add ddH20 to all vials.
Allow the solid to dissolve before inverting.
*Add 1 mL ddH20 to Standard A
*Add 0.5 mL ddH20 to Standard B-F and Controls.
Mix thoroughly by inversion prior to use but avoid foam.

Load assay tubes. Time: _________

Do not add anything to TC tubes 1, 2

Pipette 25μL of Standard A into tubes 3, 4
"     Standard B into tubes 5, 6
"     Standard C into tubes 7, 8
"     Standard D into tubes 9, 10
"     Standard E into tubes 11, 12
"     Standard F into tubes 13, 14

Pipette 25μL of Level I control to tubes 15, 16
"     Level II control to tubes 17, 18
"     WISER Control I to tubes 19, 20
"     WISER Control II to tubes 21, 22

Pipette 25μL of Unknown samples (in duplicate) to tubes 23+

Immediately add 500 μL Progesterone [I-125] to all tubes. Time: __________
Use a repeating pipette in position 2 and a 12.5mL pipette tip.

Mix by shaking the test tube rack gently by hand.
Incubate all tubes at 37 °C for 60-70 minutes on the water bath. Time: _________

Remove TC tubes and set aside. Place remaining tubes in a sponge rack and decant by simultaneous inversion into a radioactive waste receptacle. Strike the tubes sharply on absorbent material to facilitate complete drainage and then allow them to drain for 3 minutes. Blot the tubes to remove any droplets adhering to the rim before returning them to the upright position. Time: _________

Return TC tubes back into the test tube rack.

Count all tubes in the Gamma counter (1 min):
- Call lab 4-5348
- Take floppy diskette
- Sign-in sheet date, your initials, Kurzer, # tubes
- Insert diskette
- Drawer under the gamma counter contains protocol clips
- Place # 36 clip on left side of 1st tray (1st tray is furthest from you)
- Load tubes in trays from right to left
- Do not load any tubes in stop rack
- Place the stop rack behind all of your trays
- Make sure trays are perpendicular and notch is in the tray groove
- Use function keys to run protocol
- Remove clip from tray and place in numerical order in drawer
- Eject diskette
- Get disk, printout, and make sure area is left clean and organized
- If anything goes awry contact Alma (lab) 5-8292 or (cell) 909-964-7283 and Wanda (o) 5-1249, (barn) 4-3062
TESTOSTERONE RIA
DSL-4100

Testosterone RIA
Date of assay: ____________
Time samples thawed: __________
Time RIA kit to room temp: _______ (leave precipitating reagent in refrigerator)

Allow all reagents to reach room temperature.

Label and arrange 12x75mm plastic tubes in duplicate for Total Counts, Non-Specific Binding (NSB), Standards, Controls, and Unknowns (samples).

Reconstitute Standards and Controls using ddH2O.
Use same pipette tip to add ddH2O to all vials.
Allow the solid to dissolve before inverting.
*Add 1 mL ddH2O to Standard A; invert to mix.
*Add 0.5 mL ddH2O to Standard B-F and Controls.
Mix thoroughly by inversion prior to use but avoid foam.

Load assay tubes. Time: __________

Do not add anything to TC tubes 1, 2

Pipette 150 µL of Standard A (0 pg/mL) into NSB tubes 3, 4

Pipette 50 µL of Standard A into tubes 5, 6
“ Standard B into tubes 7, 8
” Standard C into tubes 9, 10
” Standard D into tubes 11, 12
” Standard E into tubes 13, 14
” Standard F into tubes 15, 16

Pipette 50 µL of Level I control to tubes 17, 18
“ Level II control to tubes 19, 20
WISER Control I to tubes 21, 22
WISER Control II to tubes 23, 24

Pipette 50 µL Unknown samples (in duplicate) to tubes 25+

Add 500 µL of the Testosterone [I-125] Reagent to all tubes. Time: __________
Use a repeating pipette in position 2 and a 12.5mL pipette tip.

Add 100 µL of the Testosterone Antiserum to all tubes except NSB and TC tubes (1-4). Time: __________ Use a repeating pipette in position 2 and a 2.5mL pipette tip.
Cover all the tubes with paraffin and gently vortex them (level 4, ~30 sec): Time: 

Incubate all test tubes at 37 °C for 60-70 minutes in the water bath. Time: 

Allow Precipitating Reagent to reach room temp. Time: 

Shake reagent thoroughly

Add 1.0 mL of Precipitating Reagent (shake thoroughly) to all tubes except TC. Time: 

Use a repeating pipette in position 4 with a 12.5 mL pipette tip.

Cover test tube rack with paraffin, vortex and incubate at room temperature for 10-15 minutes. Time: 

Centrifuge all tubes except TC 15-20 minutes at 3000 rpm

Decant all tubes except TC:
- Placing all tubes into a sponge rack
- Inverting sponge rack into radioactive waste container
- Allowing tubes to drain on chux for 1-2 minutes
- Gently blotting droplets on rims
- Returning to upright position
- Allowing to dry upright for 1-2 hours

Return TC tubes back into the test tube rack.

Count all tubes in the Gamma counter (1 min):
- Call lab 4-5348
- Take floppy diskette
- Sign-in sheet date, your initials, Kurzer, # tubes
- Insert diskette
- Drawer under the gamma counter contains protocol clips
- Place #12 clip on left side of 1st tray (1st tray is furthest from you)
- Load tubes in trays from right to left
- Do not load any tubes in stop rack
- Place the stop rack behind all of your trays
- Make sure trays are perpendicular and notch is in the tray groove
- Use function keys to run protocol
- Remove clip from tray and place in numerical order in drawer
- Eject diskette
- Get disk, printout, and make sure area is left clean and organized
- If anything goes awry contact Alma (lab) 5-8292 or (cell) 909-964-7283 and Wanda (o) 5-1249, (barn) 4-3062
APPENDIX A-2

ELISA METHOD
SHBG ELISA
IBL-59106

Date of assay: ____________
Time samples thawed: ______________
Time kit to room temp: ____________

Allow all reagents to reach room temperature and mix liquid reagents thoroughly by gentle inversion before use.

Plasma Sample Dilution: Plasma samples (Unknowns) must be diluted 1:10 with Calibrator A prior to assay (for example, 10 µL serum + 90 µL Calibrator A). Do not dilute Standards and Controls.

Prepare Wash Solution: Dilute Wash Buffer Concentrate 1:10 with distilled water into a plate washer buffer bottle (for example: one 50mL bottle of wash buffer concentrate with 450mL of distilled water, per kit). The Wash Solution is stable for one month at room temperature.

Prepare Conjugate Working Solution: Dilute anti-SHBG monoclonal antibody-HRP conjugate 1:50 in assay buffer (for example, one 300µL bottle of HRP in 15mL of assay buffer, per kit).

Load microplate wells:

Pipe 20 µL of Standard A into wells A1, A2
“ Standard B into wells B1, B2
” Standard C into wells C1, C2
“ Standard D into wells D1, D2
” Standard E into wells E1, E2
” Standard F into wells F1, F2
Pipe 20 µL Kit Control into wells G1, G2
Pipe 20 µL Internal Control into wells H1, H2

Pipe 20 µL diluted samples (in duplicate) to A3-A4 down to H3-H4 and then moving to the next set of wells to the right: PLATE 1: ________________
PLATE 2: ________________

Add 200 µL of Assay Buffer to each well using a multi-channel pipettor:

Incubate the plates on an orbital microplate shaker at 250 rpm for 30 minutes: PLATE 1: ________________
PLATE 2: ________________
While shaking, prepare the Conjugate Working Solution: one 300μL bottle of HRP in 15mL of assay buffer, per kit.

Aspirate and Wash each well 3 times with the Wash Solution using an automatic microplate washer.

**TO USE MICROPLATE WASHER ELx405:** Attach Wash Solution to intake hose. Make sure all other hoses are connected correctly to waste bottles and vacuum pump. Prime the washer by selecting RUN → PRIME → P_DAY PRIME → ENTER →START. Then place the plate with the samples in the washer, press RUN → WASH → SHBG → ENTER → START.

Add **150 μL** of the Conjugate Working Solution to each well using a multi-channel pipettor:

Incubate the wells on an orbital microplate shaker at 250 rpm for **15 minutes**:

<table>
<thead>
<tr>
<th>Plate</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLATE</td>
</tr>
<tr>
<td>2</td>
<td>PLATE</td>
</tr>
</tbody>
</table>

Aspirate and Wash each well 3 times with the Wash Solution using the ELx405 SHBG wash program.

Add **150 μL** of the TMB substrate to each well using a multi-channel pipettor:

Incubate the wells on an orbital microplate shaker at 250 rpm for **10-15 minutes**:

<table>
<thead>
<tr>
<th>Plate</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLATE</td>
</tr>
<tr>
<td>2</td>
<td>PLATE</td>
</tr>
</tbody>
</table>

Add **50 μL** of the Stopping Solution to each well using a multi-channel pipettor:

<table>
<thead>
<tr>
<th>Plate</th>
<th>Stopping Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLATE</td>
</tr>
<tr>
<td>2</td>
<td>PLATE</td>
</tr>
</tbody>
</table>

Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.

Results are given in nmol/L.
LC-MS/MS METHOD FOR ESTROGEN METABOLITE EXCRETION
URINE SAMPLE PREPARATION

Day 1
1. Thaw urine samples previously frozen and preserved in sodium azide and/or sodium ascorbate
2. Mix thoroughly by vortex to ensure homogeneity
3. Centrifuge samples for 5 minutes at 5°C
4. Pipette 1.0 ml of urine into clean, SILANIZED glass, screw-top test tubes
5. To each aliquot of urine, add 10 μl of deuterated-EM working internal standard solution (ISS) containing representative analytes of each compound
6. Following the addition of ISS, add 1.0 ml of freshly prepared enzymatic Hydrolysis Buffer pH 4.1. This buffer contained 2.0 mg of L-ascorbic acid, 5 μl of α-Glucoronidase/Sulfatase from Helix Pomatia (Type H-2), and 1.0 ml of Sodium acetate.
7. Vortex samples using batch shaker for about 1 minute
8. Incubate samples overnight at 37 °C to ensure hydrolysis

Do the following steps in the hood
9. Remove hydrolyzed samples from the 37 °C incubator and allow to reach room temperature
10. Extract the samples by adding 3 ml of ethyl ether to each tube
11. Cap and vortex on batch shaker for 1 minute
12. Centrifuge for 5 minutes at 5°C to allow phase separation
13. Pipette upper ethyl ether layer into a clean silanized glass, screw-top test tube. Be careful not to transfer any aqueous or interfacial layer to the receiving tube as this may adversely impact later derivatization.
14. Repeat this extraction steps 2-5 three times.
15. Pool ethyl ether extracts in the same tube
16. Evaporate to dryness at 40 °C with nitrogen
17. If you plan to store and freeze at this point, bring back sample in 0.15 ml of methanol and transfer to sample vials with fused low volume SILANIZED inserts.
18. If you plan to run on LC/MS the same day, take samples to the UMCC Mass Spec Lab. To the dried samples, add 25 μl of 0.1M sodium bicarbonate buffer (pH 9; 0.1% ascorbic acid).
19. Add 25 μl of dansyl chloride solution (1mg/ml acetone) which is the derivatizing solution
20. Vortex samples
13. Heat samples for 6 minutes at 60 °C to fully derivatize (allow EM and d-EM dansyl derivatives to form).
14. Analyze samples on Thermo Electron Quantum Discovery Max Triple Quadrupole LC-MS/MS Instrument.
15. Use Thermo Electron Xcalibur proprietary software for quantitative analysis

The chromatographic separation was performed on a 100 x 0.5mm (i.d) Zorbax SB-C18 column 1.8μm particle size.

The mobile phase consisted of two eluents; solvent A (50 ml/L acetonitrile-950ml/L H2O containing 1mL/L formic acid) and solvent B (950 ml/L acetonitrile-50 ml/L H2O containing 1 ml/L formic acid).

Calibration standards and quality control samples were similarly hydrolyzed, extracted, and derivatized.

**STANDARD PREPARATION**

1. Prepare the working **EM Standard Solution (EMSS)** containing the 12 non-deuterated EM to a concentration of 1 ng/μl (i.e., 1 ppm).
2. Prepare the working **Internal Standard Solution (ISS)** containing the 7 deuterated EM to get a concentration of 1 ng/μl (i.e., 1 ppm).
3. Prepare serial standard dilution of the stock solutions:
   a. **Dilution Tube 1** – Add 100μl of EMSS and 900μl of methanol and vortex. This tube contains 100ng/mL of standard and is labeled “Std 100”
   b. **Dilution Tube 2** – Add 100μl of EMSS and 2.90ml of methanol and vortex. This tube contains 100ng in 2.9 ml of methanol to give a final concentration of 33.33 ng/ml
   c. **Dilution Tube 3** – Pipette 2 ml of methanol to each tube
   d. Pipette out 1ml of **Dilution Tube 2** into a clean, silanized glass tube labeled “Std 33” and another 1 ml into **Dilution Tube 3**. Discard remaining 1 ml of **Dilution Tube 2**.
   e. Gently but thoroughly vortex the contents of **Dilution Tube 3**. This tube contains 11.11ng/ml of standard. Transfer 1 ml of **Dilution Tube 3** into a clean silanized tube labeled “Std 11” and another 1 ml into **Dilution Tube 4**.
   f. Gently but thoroughly vortex the contents of **Dilution Tube 4**. This tube contains 3.704ng/ml of standard. Transfer 1 ml of **Dilution Tube 4** into a clean silanized tube labeled “Std 3.7” and another 1 ml into **Dilution Tube 5**.
   g. Gently but thoroughly vortex the contents of **Dilution Tube 5**. This tube contains 1.23ng/ml of standard. Transfer 1 ml of **Dilution Tube 5** into a
clean silanized tube labeled “Std 1.23” and another 1 ml into Dilution Tube 6.

h. Gently but thoroughly vortex the contents of Dilution Tube 6. This tube contains 0.412ng/ml of standard. Transfer 1 ml of Dilution Tube 6 into a clean silanized tube labeled “Std 0.41” and another 1 ml into Dilution Tube 7.

i. Gently but thoroughly vortex the contents of Dilution Tube 7. This tube contains 0.137ng/ml of standard. Transfer 1 ml of Dilution Tube 7 into a clean silanized tube labeled “Std 0.14”

4. Add 10μl of 1ppm ISS (equivalent to 10 ng) to each standard tube

5. Blow dry in nitrogen
APPENDIX B

WISER STUDY HANDBOOK
# WISER Participant Handbook

## Table of Contents

### Overview

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline of participation</td>
<td>141</td>
</tr>
</tbody>
</table>

### General Data Collection Information

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fitness Assessments</td>
<td>149</td>
</tr>
<tr>
<td>Blood Draws</td>
<td>149</td>
</tr>
<tr>
<td>DEXA Scans</td>
<td>150</td>
</tr>
<tr>
<td>Health Surveys</td>
<td>150</td>
</tr>
</tbody>
</table>

### Data Collection for Participants

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keeping a Menstrual Log</td>
<td>151</td>
</tr>
<tr>
<td>Using the Ovulation Predictor Kits</td>
<td>151</td>
</tr>
<tr>
<td>Completing the 3-day Urine Collections</td>
<td>153</td>
</tr>
<tr>
<td>Completing the 3-day Sleep Journals</td>
<td>156</td>
</tr>
<tr>
<td>Completing the 3-day Food Records</td>
<td>158</td>
</tr>
<tr>
<td>Completing the Food Frequency Questionnaire</td>
<td>162</td>
</tr>
<tr>
<td>Using a Heart Rate Monitor</td>
<td>163</td>
</tr>
<tr>
<td>Completing the Exercise Training Workout Logs</td>
<td>164</td>
</tr>
</tbody>
</table>

### WISER Contact Information

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheduling Appointments and Questions</td>
<td>166</td>
</tr>
<tr>
<td>WISER Staff</td>
<td>167</td>
</tr>
<tr>
<td>General Clinical Research Center (GCRC)</td>
<td>168</td>
</tr>
</tbody>
</table>
**Data Collection Forms**

- Daily Food Record 175
- Sleep Journal 178
- Workout log 181

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*PLEASE NOTE: If at any time you have questions or problems arise, please contact any of the WISER staff immediately. Contact information is in this handbook and on the website ([www.wiserwomen.umn.edu](http://www.wiserwomen.umn.edu)). WISER staff is here to assist you through the study process. Do not hesitate to call for any reason 612-968-9589.*
### WISER Overview—Timeline of Participation

#### Menstrual Cycle 1

| Date       | 06.27.07 | 06.28.07 | 06.29.07 | 06.30.07 | 07.01.07 | 07.02.07 | 07.03.07 | 07.04.07 | 07.05.07 | 07.06.07 | 07.07.07 | 07.08.07 | 07.09.07 | 07.10.07*  | 07.11.07 | 07.12.07 | 07.13.07 | 07.14.07 | 07.15.07 | 07.16.07 | 07.17.07 | 07.18.07 | 07.19.07 | 07.20.07 | 07.21.07 | 07.22.07 | 07.23.07 | 07.24.07 | 07.25.07 | 07.26.07 | 07.27.07 | 07.28.07 | 07.29.07 | 07.30.07 |
|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Day of Cycle | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
| Flo’s Menstrual Cycle | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Menstruation begins (Cycle 1) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ovulation Predictor Kit | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DO NOT EXERCISE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DO NOT EXERCISE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clinic Visit 1 (~60 minutes) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Clinic Visit 1:** will be scheduled 8 days after a positive Ovulation Predictor Kit test.

- Contact WISER Staff—**upon a positive Ovulation Predictor Kit test**—at (w) 612.968.9589 (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for 8 days after positive ovulation predictor kit test (if day 8 falls on a Saturday Clinic Visit 1 will be Friday and if day 8 falls on Sunday Clinic Visit 1 will be Monday)

If leaving a message, give:
- Your name and contact information, along with what early morning times you are available on day 8 for your clinic visit (try to give a range of times, and what would be best. For example, “I am free 8-10, but would prefer 8:30.”) **
  *Remember that every following clinic visit will have to be within one hour of this time.**
- Discontinue ovulation predictor testing after a positive result
- DO NOT exercise 48 hours prior to visit
- Do not eat anything or drink anything but water 10 hours before clinic visit—e.g. if clinic visit 1 is at 7:30 AM do not eat anything but water after 9:30 PM the night before (Clinic Visit 1 should be scheduled during early morning hours)

### Menstrual Cycle 2

| Date    | 07.31.07 | 08.01.07 | 08.02.07 | 08.03.07 | 08.04.07 | 08.05.07 | 08.06.07 | 08.07.07 | 08.08.07 | 08.09.07 | 08.10.07 | 08.11.07 | 08.12.07 | 08.13.07 | 08.14.07 | 08.15.07 | 08.16.07 | 08.17.07 | 08.18.07 | 08.19.07 | 08.20.07 | 08.21.07 | 08.22.07 | 08.23.07 | 08.24.07 | 08.25.07 | 08.26.07 | 08.27.07 | 08.28.07 | 08.29.07 | 08.30.07 | 08.31.07 | 09.01.07 | 09.02.07 |
|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Day     | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        | 9        | 1        | 0        | 1        | 1        | 2        | 1        | 3        | 4        | 1        | 5        | 1        | 6        | 1        | 7        | 1        | 8        | 1        | 9        | 2        | 0        | 2        | 1        | 2        | 3        | 2        | 4        | 2        | 5        | 2        | 6        | 2        | 7        | 2        | 8        | 2        | 9        | 3        | 0        | 3        | 3        | 3        | 3        | 4        |
| Cycle   |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |

<table>
<thead>
<tr>
<th>Flo's Menstrual Cycle</th>
<th>Menstruation begins (Cycle 2)</th>
<th>Begin urine collection &amp; food diary</th>
<th>Clinic Visit 2 (~ 60 minutes)</th>
<th>Last day of urine collection &amp; food diary</th>
<th>Establish urine &amp; diary pick-up</th>
<th>FITNESS ASSESSMENT</th>
<th>Ovulation Predictor Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>
Clinic Visit 2: will be scheduled on day 8 of menstrual cycle 2.
  - Contact WISER Staff—on day 1 of menstrual cycle 2—at (w) 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for day 8 of menstrual cycle 2 (if day 8 falls on a Saturday Clinic Visit 2 will be Friday and if day 8 falls on Sunday Clinic Visit 2 will be Monday) **Time of visit should be scheduled ± 1 hour of Clinic Visit 1.**
  - If leaving a message, give:
    - Your name and contact information, along with what early morning times you are available on day 8 for your clinic visit (try to give a range of times, and what would be best. For example, “I am free 8-10, but would prefer 8:30.”)
    - Do not put off calling after you get a positive --- feel free to leave a message with either of her phone or email!
  - DO NOT exercise 48 hours prior to visit
  - Start urine collection the morning of day 7 (the day before Clinic Visit 2)
  - Do not eat anything or drink anything but water 10 hours before clinic visit—e.g. if clinic visit 2 is at 7:30 AM do not eat anything but water after 9:30 PM the night before  (Clinic Visit 2 should be scheduled during early morning hours)
  - You will learn which group you have been randomized into at the fitness assessment

Menstrual Cycle 2—continued

Day 10 Urine Pick up: will be scheduled for day 10 of menstrual cycle 2.
  - WISER staff will contact you regarding the urine pick-up details. If you don’t hear from someone by your 2nd clinic visit please let WISER staff know.
  - Provide urine jugs and food diary for pick-up staff.

Day 11 Fitness Assessment: will be on day 11 of menstrual cycle 2.
  - BETH KAUFMAN, AMANDA THIESCHAFTER, HOLLY JAKITS- WISER Personal Trainers, will contact you to schedule a fitness assessment.
  - If randomized into exercise group, you will discuss exercise program with trainer at fitness assessment.
Menstrual Cycle 3

| Date   | 09.03.07 | 09.04.07 | 09.05.07 | 09.06.07 | 09.07.07 | 09.08.07 | 09.09.07 | 09.10.07 | 09.11.07 | 09.12.07 | 09.13.07 | 09.14.07 | 09.15.07 | 09.16.07 | 09.17.07 | 09.18.07 | 09.19.07 | 09.20.07 | 09.21.07 | 09.22.07 | 09.23.07 | 09.24.07 | 09.25.07 | 09.26.07 | 09.27.07 | 09.28.07 | 09.29.07 | 09.30.07 | 10.01.07 | 10.02.07 | 10.03.07 | 10.04.07 |
|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Day of Cycle | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 3 | 1 | 4 | 1 | 5 | 1 | 6 | 1 | 7 | 1 | 8 | 1 | 9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

Flo’s Menstrual Cycle

<table>
<thead>
<tr>
<th>Flo’s Menstrual Cycle</th>
<th>Menstruation begins (Cycle 3)</th>
<th>Clinic Visit 3 (~15 minutes)</th>
<th>Ovulation Predictor Kit</th>
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</thead>
<tbody>
<tr>
<td>09.03.07</td>
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</table>

**Clinic Visit 3:** will be scheduled on **day 10 of menstrual cycle 3**.

- Contact WISER Staff—on **day 1 of menstrual cycle 3**—at (w) 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for day 10 of menstrual cycle 3 **Time of visit should be scheduled ± 1 hour of Clinic Visit 1.**
- Clinic Visit 3 is for **weight check only**—in light clothing, no shoes—while we understand there are variations in body weight we are asking that you try to maintain your weight (± 5 pounds) throughout study participation.
- **Do not put off calling** after you get a positive --- feel free to leave a message with either of her phones or email!
Menstrual Cycle 4

Clinic Visit 4: will be scheduled on day 10 of menstrual cycle 4.

- Contact WISER Staff—on day 1 of menstrual cycle 4—at 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for day 10 of menstrual cycle 4. **Time of visit should be scheduled ± 1 hour of Clinic Visit 1.
- Clinic Visit 4 is for weight check only— in light clothing, no shoes—while we understand there are variations in body weight we are asking that you try to maintain your weight (± 5 pounds) throughout study participation
- Remember to bring your menstrual logs to the clinic for staff to check off.
- Do not put off calling after you get a positive --- feel free to leave a message with either of her phones or email!
Menstrual Cycle 5

| Date       | 11.05.07 | 11.06.07 | 11.07.07 | 11.08.07 | 11.09.07 | 11.10.07 | 11.11.07 | 11.12.07 | 11.13.07 | 11.14.07 | 11.15.07 | 11.16.07 | 11.17.07 | 11.18.07 | 11.19.07 | 11.20.07 | 11.21.07 | 11.22.07 | 11.23.07 | 11.24.07 | 11.25.07 | 11.26.07 | 11.27.07 | 11.28.07 | 11.29.07 | 11.30.07 | 12.01.07 | 12.02.07 | 12.03.07 | 12.04.07 | 12.05.07 | 12.06.07 | 12.07.07 |
|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Day of Cycle | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 |
| Flo’s Menstrual Cycle | Menstruation begins (Cycle 5) | Ovulation Predictor Kit | Clinic Visit 5 (~60 minutes) | DO NOT EXERCISE | DO NOT EXERCISE |

**Clinic Visit 5:** will be scheduled 8 days after a positive Ovulation Predictor Kit test.

- Call WISER Staff when you get your Day 1 for our records.
- Next time to contact WISER Staff—**upon a positive Ovulation Predictor Kit test**—at (w) 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for 8 days after positive ovulation predictor kit test (if day 8 falls on a Saturday Clinic Visit 5 will be Friday and if day 8 falls on Sunday Clinic Visit 5 will be Monday) **Time of visit should be scheduled ± 1 hour of Clinic Visit 1.**

If leaving a message, give:
Your name and contact information, along with what early morning times you are available on day 8 for your clinic visit (try to give a range of times, and what would be best. For example, “I am free 8-10, but would prefer 8:30.”)

- Discontinue Ovulation Predictor Kit testing after a positive result
- DO NOT exercise 48 hours prior to visit
- Do not eat anything or drink anything but water 10 hours before clinic visit—e.g. if clinic visit 5 is at 7:30 AM do not eat anything but water after 9:30 PM the night before (Clinic Visit 5 should be scheduled during early morning hours)

### Menstrual Cycle 6

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<td>Day of Cycle</td>
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<tr>
<td>Flo’s Menstrual Cycle</td>
<td>Menstruation begins (Cycle 6)</td>
<td>Stop exercise (P/A Group)</td>
<td>Begin urine collection &amp; food diary</td>
<td>Clinic Visit 6 (~60 minutes)</td>
<td>Last day of urine collection &amp; food diary</td>
<td>Establish urine &amp; food diary collection</td>
<td>FITNESS ASSESSMENT</td>
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Clinic Visit 6: will be scheduled on day 8 of menstrual cycle 6.

- Contact WISER Staff—on day 1 of menstrual cycle 6—at (w) 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with GCRC for day 8 of menstrual cycle 6 (if day 8 falls on a Saturday Clinic Visit 6 will be Friday and if day 8 falls on Sunday Clinic Visit 6 will be Monday) **Time of visit should be scheduled ± 1 hour of Clinic Visit 1.

If leaving a message, give:
- Your name and contact information, along with what early morning times you are available on day 8 for your clinic visit (try to give a range of times, and what would be best. For example, “I am free 8-10, but would prefer 8:30.”)
- DO NOT exercise 48 hours prior to visit
- Start urine collection the morning of day 7 (the day before Clinic Visit 6)
- Do not eat anything or drink anything but water 10 hours before clinic visit—e.g. if clinic visit 6 is at 7:30 AM do not eat anything but water after 9:30 PM the night before (Clinic Visit 6 should be scheduled during early morning hours)

Menstrual Cycle 6—continued
- Bring completed menstrual log to Clinic Visit 6

Day 10 Urine Pick up: will be scheduled for day 10 of menstrual cycle 6.
- WISER staff will contact you be contacted from WISER staff regarding the urine pick-up details. If you don’t hear from someone by your 2nd clinic visit please let WISER staff know.
- Provide urine jugs and food diary for pick-up staff.

Day 11 Fitness Assessment: will be on day 11 of menstrual cycle 6.
- BETH KAUFMAN, HOLLY JAKITS or AMANDA THIESCHAFER, WISER Personal Trainers, will contact you to schedule a fitness assessment.

If randomized into exercise group, will discuss exercise program with trainer at fitness assessment.
General Data collection information

Fitness Assessments

On or about day 11 of your 2nd and 6th cycles, you will walk on a treadmill with a heart rate monitor at 3.5 miles per hour with gradual increase in the incline of the treadmill until you reach a heart rate of 85% of your age predicted maximal heart rate (total time: 15 minutes). Fitness assessments at baseline and follow-up allow us to evaluate participants’ cardiorespiratory endurance. This fitness assessment will take place at the University of Minnesota Recreational Center on the East Bank.

There is the possibility of muscle soreness from exercise during the fitness assessments on day 11 of menstrual cycles 2 and 6 or during the exercise intervention. This soreness may last several days after each exercise training session, but it is not likely to be severe enough to limit any of your usual daily activities. There is also a small risk of muscle injury from exercise. Muscle injuries may require medical attention, may take several months to heal, and may limit your usual daily activities for a period of days or weeks.

Blood Draws

During 4 clinic visits you will have 2 tablespoons of blood drawn each time. Blood draws have to occur after 10+ hours of fasting and are therefore scheduled during the early morning hours. Blood is drawn to measure the following biomarkers at baseline and follow-up: estrogen and progesterone hormones, oxidative stress, inflammatory markers, and insulin and glucose.

If you choose to participate in the optional DNA study, 2 additional tablespoons will be drawn at Clinic Visit 1 only.

There is a small risk of infection when blood is taken, but the risk is minimal as all needles and equipment are sterilized and the procedures are performed by clinical staff at the General Clinical Research Center. You may experience some mild to moderate pain lasting a few seconds upon insertion of the needle used to draw the blood. You may also get a bruise from the blood draw.
DEXA Scans

During 2 clinic visits body composition will be assessed using the dual x-ray absorptiometry (DEXA) machine. DEXA scans are full-body x-rays done while lying down. You will need to remove all jewelry prior to having the DEXA scan.

Note that pregnant women are excluded from this study, and that if you become pregnant, it will be important to inform the study investigators. To ensure no unborn fetus is exposed to radiation, we conduct the pregnancy test with each participant before proceeding with the DEXA scan. The risks associated with exposing a fetus to ionizing radiation through this DEXA procedure are greater than those associated with exposing adults.

Although it is possible to estimate body fat by taking a measure of a fold of your skin (with a skin caliper tool), the margin of error with that kind of measurement is much larger than for DEXA. It is so much larger that we would need to double our sample size in order to detect any significant changes in body fat percentage. The measure of body fat we obtain from DEXA, a low risk and reliable procedure, improves our ability to test the study hypothesis.

Health Surveys

There are several health surveys being administered at baseline and again at follow-up. The surveys include a physical activity interview along with a survey asking questions regarding the following: exercise, life events, emotional state, body image, menstrual cycle characteristics, and demographics.
Data Collection for Participants

Keeping a Menstrual Log

You will be given a monthly calendar for the months you participate in the project. The calendar should be used for tracking your menstrual cycle—"start of menstruation," menstrual flow, "end of menstruation," signs and symptoms of menstruation, ovulation test results, + ovulation test date, and length of menstrual cycle—along with urine collection dates, food record dates, and scheduled clinic visits at the General Clinical Research Center.

Remember to note the following:
- **Start of menstruation**, time of day, cycle number, and flow perception (*P#/AM/Moderate/Anxiety, Cramps). Where period flow is categorized as:
  - Very heavy, heavy, moderate, somewhat light, or very light
- And symptoms include:
  - Acne, anxiety, backache, bloating, breast swelling, breast tenderness, cramps, fatigue, food craving, headache, irritability, joint pain, moody, muscle pain/aches, nausea, tension, water retention, or weight gain
- Urine collection dates (urine)
- Food record dates (fd rec)
- Scheduled clinic visits with GCRC (CV1)
- Urine pick-up (PU)
- **Ovulation test results** (- OVU or + OVU)
- **Last day of menstrual flow/period** (**)P# End**
- Number of days in menstrual cycle (+1, +2, … +28)

Please see pages 15-20 for a sample of a completed menstrual log

Using the Ovulation Predictor Kits

The ovulation kits will be used during five menstrual cycles, starting with your first menstrual cycle. You will be given a nine-day ovulation kit to begin use on or about day 10 of your menstrual cycle. The first day of Ovulation Predictor Kit testing should occur according to your shortest cycle length (e.g. Flo’s average cycle length is 29 days; her shortest cycle is 27 days. Flo will start Ovulation Predictor tests on day 10 of her cycle.). You may take the test at any time of the day you find convenient. However, you should take the test at approximately the same time each day and mid-afternoon is best.
You should avoid consuming excess amounts of liquid for at least 4 hours prior to performing the test.

How to perform the test:
1. Collect urine using the urine cup provided. If the test will be carried out after more than 1 hour from the time of urine collection, the urine should be refrigerated. If refrigerated, urine should be allowed to warm to room temperature before testing.
2. When you are ready to begin the test, open one of the test pouches (open one at a time, immediately before testing). Remove the device from the foil.
3. Draw urine up to the marked line on the pipette provided (approximately 5 drops). Disperse entire contents of the pipette into the sample well. Wait 3 minutes for the result to develop.
4. Read the results between 3 – 5 minutes later.

Interpretation of the results:
1. If there is no line in the test region (T), or the line is lighter than the one in the control region (C), then you have not begun your LH surge, and you should continue to test daily.
2. If the color of the line in the test region is equivalent to or more intense than the line in the control region, you have detected a positive Ovulation Predictor test. Ovulation should occur within 24-36 hours.
3. During Menstrual Cycles 1 and 6: Contact WISER Staff at (w) 612.968.9589 or (e) wiser@umn.edu to assist in scheduling an appointment with the GCRC for 8 days after a positive Ovulation Predictor test.

Do not put off calling WISER Staff after you get a positive --- feel free to leave a message with either of her phones or email!
Completing the 3-day Urine Collections

*** Please call if this is confusing at all: 612-624-3050 or 612-968-9589***

The urine collections will be two separate 72-hour collections, the first taken for three consecutive days near the beginning of your second menstrual cycle (days 7, 8, 9) and then once again for three days during your sixth menstrual cycle (days 7, 8, 9).

The GCRC will provide you with four large 3-liter containers and four 1-liter containers, one set of large and small containers for each day you collect urine and one additional set (in case you fill one on any given day and need another). For each collection day the urine produced during the day will be put in the large 3-liter jug and the overnight urine starting at 11:00 pm will be put in the smaller 1-liter jug, including the first morning void of the following day. Note that the overnight urine should be kept separately from the urine produced during the previous day, this means that for each 24 hours there will be one 3-liter container with daytime urine and one 1-liter container with nighttime urine, including the first morning void.

You will be provided with 4 baggies containing vitamin C and the contents of 1 baggie of powdered vitamin C must be put in each large 3-liter jug, which helps to preserve the urine. Note that the overnight urine stored in the 1-liter containers should not have vitamin C in it. The extra 1-liter collection container and accompanying cooler bag with the ice packs are meant to accommodate your active lifestyles of work, school and other commitments.

You will also be provided with two collection hats. They are designed to make the collections easier. You can keep one at work and one at home if you like. Place the hat near the front of the toilet bowl before you urinate. After you urinate, pour the urine into the container.

Each day, you will need to clean the 1-liter container and the collection hat. Wash them with hot water and dish soap. Make sure the liter container is dry before you use it again.

Please follow the instructions below to complete the 3-day urine collections.

1. On your first urine collection day, do not collect your first void in the morning. That urine was produced in your body overnight and therefore is representative of the previous night's urine. At this point, with the 3-liter jugs still empty, add the vitamin C powder to each container.

2. Record the time of that first void of the day as your starting time on the label and in your menstrual calendar. Urine is being produced in your body from that time on and will be collected in the first day's collection. So although you are not collecting this first void, it is the start time for the first 24 hours of the collection period.
NOTE: someone will contact you to schedule a time for pick up on day 10. WISER staff will verify that you have arranged a pick-up at the second clinic visit.

3. Throughout the day, whenever you are away from home, carry the 1-liter container and collection hat with you in the cooler bag with ice packs to ensure a full day’s collection.

4. When you arrive home for the evening, immediately transfer the urine to the large 3-liter container labeled for that day. Each container may only contain urine from one day. Label your 3-liter urine container with the start and end times as well as the appropriate dates. You will be provided with labels to attach to all your containers. Keep all containers filled with urine in a refrigerator or cooler with ice. DO NOT STORE OUTSIDE during the winter.

5. The ‘day collection’ for day 7 finishes at 11:00 pm if you got to bed after 11:00 pm. In this case, you need to remember to void at 11:00 pm and transfer the urine to the orange jug. If you go to bed before 11:00 pm, make sure you void then, and write down the time on the label. So, if you go to bed at 9:30 pm, you should urinate and write down 9:30 pm as the end time for the daytime collection and start time for the nighttime collection.

6. When you wake up the next day (day 8), void and transfer that urine into the 1-liter container for day 7. This void constitutes the last void of the first day of urine collection. **Note that your start and end time for the 24 hour urine collections will depend on what time you get up that first morning. (The end time for the previous day is the start time for the next day.)** You will need to get up at the same time for each of the three days of the 24-hour urine collections. You will need to urinate the following morning at this same time in order to finish each day's collection period.

   EXAMPLE: On day 7, you wake up and void at 8 a.m., but you do not collect since this urine was produced in the previous 24 hours. This is your START TIME of collection day 7—8 a.m., 02-06-06 (per Flo’s example). On day 8, you wake up again at 8 a.m., void and transfer the urine into the 1-liter container labeled for the 1st day. Record the time of first voids in the menstrual log calendars.

7. Keep your 3-liter and 1-liter containers of the urine collections in the refrigerator.

8. Repeat the steps as above, for the 2nd and 3rd days of urine collection – keeping in mind that the very last void you collect will actually be the morning of day 10 and it will be put in the smaller 1-liter container corresponding to day 9.
9. A research assistant will transfer the 3-liter and 1-liter containers with the three 24-hr urine collections to the GCRC. Someone will contact you to arrange for a urine pick-up. If you do not hear from someone please tell WISER staff at your 2\textsuperscript{nd} clinic visit.

\textit{What if...}

- If you happen to urinate enough one day to more than fill the 3-liter container, simply use the extra container. Make sure to label both containers and indicate there are two containers for that day. Remember that the overnight urine starting at 11:01 pm until the first morning void is kept in the 1-liter container.

- If you find while collecting urine that you produce more than 3 liters on the first day (and this was a normal day for you), please contact the study coordinator immediately and we can provide you with additional collection containers.

- NOTE: Do not fill the container to the top, as it will increase the chance of leaking. Instead, once a container is $\frac{3}{4}$ full, move on to the second container. Always make sure you have one extra 3-liter container at home.

- We do not need to know each time you use the restroom. We are concerned with the time frame for the day you collected, which should be 24 hours.

- Make sure you place all labels on the 3-liter and 1-liter containers and check on the labels for dates, time and check the box that says “Vit C added”.
### CORRECT URINE LABELS

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<tr>
<th>DAYTIME COLLECTION</th>
<th>NIGHTTIME COLLECTION</th>
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<tbody>
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### Completing the 3-Day Sleep Journals

You will be completing a sleep journal during the two 3-day urine collections that you will do while participating in the WISER study. The sleep journal should be completed first thing in the morning of days 8, 9, and 10 of the urine collections. That means that there will be one sleep journal for each day of urine collection. Make sure you read the questions before hand so you are able to answer them accurately. Blank copies of the sleep journal can be found at the end of this handbook. The sleep journals will be picked up by the WISER staff along with the food records and the urine jugs.
Sleep Journal (example)

Person ID 1000

Menstrual cycle Day: 8  Date: 01 / 17 / 2008

A. Complete these questions first thing in the morning.

1. At what time did you go to bed and try to fall asleep last night?
   ☐ No (0)  X Yes (1)
   11:30 am X pm

2. Did you wake up during the night?
   ☐ No (0)  X Yes (1) If yes, at what time(s)?
   2:30 am

3. Did you sleep with any lights on?
   ☐ No (0)  X Yes (1)
   If yes, what kind of light? small lamp, 60-watt bulb
   For how long was the light on? 11:30pm-2:30am

4. What time did you first wake up this morning?
   6:40 am

5. Did you go back to sleep after first waking up?
   ☐ No (0)  X Yes (1)
   If yes, what time did you get up and out of bed?
   7:05 am

6. Did you take any naps yesterday?
   ☐ No (0)  X Yes (1)
   If yes, at what times?
   Nap Begin  Nap End
   5:30 am X pm  6:00 am X pm

7. How would you rate your sleep quality last night?
   1  2  3  4  5
   Very Poor Below Average Average Above Average Excellent

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173
Completing the 3-Day Food Records

Instructions for Completing an Accurate Food Record

You will be completing two 3-day food records at designated times during the study. In order to calculate your nutrient intake, a complete and accurate record must be kept of your food and beverage consumption for the three days. Please be honest. If you have any questions on how to record an item, please call us. The following are instructions for recording food intake to help you to provide accurate data.

1. Complete the food diaries for 3 consecutive days (days 7, 8 and 9) during menstrual cycles 2 and 6. Include the day of the week and date on each form.

2. Use a separate form for each day. If you need extra lines to complete one day of intake, use an additional sheet. Mark the sheets (page 1 of 1, page 1 of 2) if multiple sheets are included.

3. Carry the food record sheets with you during the day and write down the foods as you consume them.
   - All meals, snacks, and “tastes” of food and beverages must be recorded, whether they are eaten at home or away.
   - If you forget to bring the record sheets, write down what you eat on a scrap of paper or napkin.

4. Record the time you eat.

5. Record the type of food, such as 2% milk, chicken noodle soup with vegetables, red delicious apple, cinnamon raisin bagel.
   - Be as specific as possible in recording food items.
   - If the item is a brand name product, please record it.
   - If you have the label of a food, keep it and turn it in with the food record.

6. Record the amount you consume.
   - The amount you consume may be different than the amount you are served (i.e. you do not finish a portion of the meal)
   - Do not assume that you eat the portion size listed on the food label.
   - Measure food and beverage items whenever possible. List portion consumed either in weight (in grams or ounces) or in volume (in teaspoons, tablespoons or cups). You may use the following abbreviations for weight and measures:
     - tsp. = teaspoon
     - Tbsp. = tablespoon
     - oz = ounce
     - c = cup
   - List the cooked weight of foods. Most meat products lose approximately one-quarter of their weight during cooking (evaporation of water, loss of fat). Therefore, 4 ounces of raw hamburger yields a 3 ounce cooked burger. Leaner varieties of meat lose slightly less weight during cooking.
If you consume certain foods or beverages on a regular basis (such as a bowl of corn flakes, a glass of milk) measure the usual servings you consume for these common items.

7. Record how the food is prepared, such as fried in 2 Tbsp. oil, steamed, peeled. If you know the type of oil used, please record it (e.g. olive oil, safflower, corn).

8. If the food is a combination food, record all the ingredients. For example, a cheeseburger might consist of:
   - 3 oz cooked beef
   - ¾ oz slice American cheese
   - 1, 2-oz onion bun
   - 2 Tbsp. ketchup
   - 1 tsp. yellow mustard

9. Include all condiments and amounts, such as 2 tsp. sugar added to coffee, or 1 Tbsp. mayonnaise spread on a sandwich.

10. Record all beverages you consume during the day and night, including water, milk, juice, pop, diet pop, tea, coffee, etc. and the amounts in fluid ounces. (Remember that 8 fluid ounces is one cup.)

11. Include all vitamins and supplements. Record the brand and type taken. If possible, please include any label information.

12. Indicate at the bottom of each daily food record if this was a typical day in terms of food intake. If it was not a typical day, briefly explain why.

13. Be sure to give a completed copy of your food record to the research assistant when your food record and urine are collected on day 10 of menstrual cycles 2 and 6.

**Portion Sizes**
When eating away from home, use the following guide to help estimate serving sizes:

- 3 ounces of cooked meat is the size of a deck of cards or the palm of your hand
- 1 chicken thigh or ½ small breast equals 2 ounces
- 1 medium chicken leg equals 2 ounces
- 1 chicken wing equals 1 ounce
- ½ cup canned fish equals 3 ounces
- ¼ cup cooked, chopped meat equals 1 ounce
- Your thumb is the size of one ounce of cheese
- The tip of your thumb is about one teaspoon of margarine or butter
- Your closed fist is about one cup of rice, noodles, vegetables, etc.
- One closed handful (the amount you can grab in your hand with fingers almost touching your palm) is about one ounce of nuts
• One large all-you-can-grab handful is about one ounce of chips or pretzels
• If you are uncertain, estimate using familiar objects. For example, you can use a “baseball” to estimate an ice cream serving.

**Measurement conversions** may be useful for understanding food labels:

- 3 tsp. = 1 Tbsp.
- 4 Tbsp. = ¼ cup
- ½ pint = 1 cup
- 8 fluid oz. = 1 cup
- 5 ⅓ Tbsp. = ½ cup
- 4 cups = 2 pints = 1 quart
**Daily Food Record (example)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Food or Beverage Consumed</th>
<th>Amount</th>
<th>Description/Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:00 am</td>
<td>Coffee with</td>
<td>10 oz</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Granulated sugar</td>
<td>2 tsp.</td>
<td></td>
</tr>
<tr>
<td>7:00 am</td>
<td>Powder shake</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cranberry juice</td>
<td>¾ c</td>
<td>Archer farms®</td>
</tr>
<tr>
<td></td>
<td>Sugar-free cocoa mix</td>
<td>2 T</td>
<td>Carnation® rich chocolate</td>
</tr>
<tr>
<td></td>
<td>Banana</td>
<td>1 large</td>
<td></td>
</tr>
<tr>
<td>10:00 am</td>
<td>Water</td>
<td>12 oz</td>
<td></td>
</tr>
<tr>
<td>12:00 pm</td>
<td>Turkey sandwich</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Turkey lunchmeat</td>
<td>3 oz</td>
<td>Louis Rich® oven roasted</td>
</tr>
<tr>
<td></td>
<td>Whole wheat bread</td>
<td>2 slices</td>
<td>Roman Meal®, 1 oz per slice</td>
</tr>
<tr>
<td></td>
<td>Margarine</td>
<td>1 tsp.</td>
<td>Promise® low fat</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>1 leaf</td>
<td>romaine lettuce</td>
</tr>
<tr>
<td></td>
<td>Bag of Doritos</td>
<td>1 ¾ oz</td>
<td>“nacho cheesier”</td>
</tr>
<tr>
<td></td>
<td>Skim milk</td>
<td>1 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orange</td>
<td>1 medium</td>
<td>fresh</td>
</tr>
<tr>
<td>3:00 pm</td>
<td>Diet coke</td>
<td>10 oz</td>
<td>Drank most of 12 oz can.</td>
</tr>
<tr>
<td></td>
<td>Frosted cake donut</td>
<td>1</td>
<td>Fried, with 2 Tbsp frosting</td>
</tr>
<tr>
<td>8 – 5 pm</td>
<td>Water</td>
<td>20 oz</td>
<td>Water consumed at desk</td>
</tr>
<tr>
<td>7:00 pm</td>
<td>Pasta with meat sauce</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spaghetti noodles</td>
<td>1 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>3 oz</td>
<td>Cooked, drained, rinsed</td>
</tr>
<tr>
<td></td>
<td>Pasta sauce</td>
<td>½ c</td>
<td>Healthy Choice® mushroom</td>
</tr>
<tr>
<td></td>
<td>Parmesan cheese</td>
<td>1 T</td>
<td>Kraft®</td>
</tr>
<tr>
<td></td>
<td>Tossed salad</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iceberg lettuce</td>
<td>2 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cherry tomatoes</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shredded carrot</td>
<td>¼ c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Italian dressing</td>
<td>2 T</td>
<td>Wine vinegar, olive oil, pinch salt</td>
</tr>
<tr>
<td></td>
<td>Mineral water with</td>
<td>12 oz</td>
<td>Mendota® Springs</td>
</tr>
<tr>
<td></td>
<td>Lime slice</td>
<td>½” slice</td>
<td></td>
</tr>
<tr>
<td>8:00 pm</td>
<td>Water</td>
<td>8 oz</td>
<td></td>
</tr>
</tbody>
</table>

**Typical day**

**X**  Not a typical day (please explain why): Was a weekend day and I was out of town.
Instructions for Completing the Food Frequency Questionnaire
You will be completing a web-based diet history questionnaire at the beginning of the study. This questionnaire consists of questions about consumption of 120 food items and it takes approximately 50-60 minutes to complete it.

1. Open your internet browser and type in the following address: https://riskfactor.cancer.gov/respondent.html
   You will see a login screen that asks for the study code, respondent id, and password. This information will be given to you in an email reminder prior to your first clinic visit.

2. Type in the study code, respondent id, and password provided. This will take you to a welcome screen, where you will click on the green button that says “Start Questionnaire”.

3. A new screen will pop up with instructions. Please read them carefully before clicking on “Start Questionnaire”.

4. Read each question carefully and follow the instructions until you are finished.

5. You may interrupt the questionnaire at any time by clicking on the log out link on the top left corner of your screen. When you log back in, you will be able to continue answering the remaining questions without having to go back to the beginning of the questionnaire.

*Please let us know if you do not have access to a computer!

Questions? Any questions about completing the food record can be directed to ANDREA at (w) 612.624.3050 or (e) plat0072@umn.edu.
Using a Heart Rate Monitor

How to use a Polar FS1 Heart Rate Monitor
To measure your heart rate you need to wear the transmitter.
Check that you are not near other people with heart rate monitors or any source of electromagnetic disturbances.

1. Attach one end of the transmitter to the elastic strap.

2. Adjust the transmitter’s strap length to fit snugly and comfortably. Secure the strap around your chest.

3. Lift the transmitter off your chest and **moisten the two grooved electrode areas on the back**. Check that the wet electrode areas are firmly against your skin and that the Polar logo is in a central, upright position.

4. Wear the wrist unit as a watch.

5. In the Time of Day display press the front button to enter the menu. **EXE** (Exercise) is displayed. *(NOTE: This will be done after completing your warm-up. You will be collecting data during your 30 minutes of aerobic exercise only.)*

6. After three seconds the wrist unit goes into Exercise mode and the stopwatch starts. The stopwatch is displayed and the outline of the heart symbol will flash until your heart rate is detected.

7. Your heart rate and the symbol will appear within 15 seconds.

8. Begin exercising immediately after seeing the heart rate symbol appear on the watch.

A flashing heart symbol indicates an ongoing heart rate measurement. The heart symbol flashes at the pace of your heart.

**Note:**
- Please allow sufficient time for signal pick up.
- If the wrist unit does not receive your heart rate, the stopwatch keeps running and the flashing heart frame symbol disappears, check that the transmitter electrodes are wet and that the strap is snug enough.
- Keep in mind that your watch saves limited amount of workouts; you will be exchanging it weekly with your trainer.
Completing the Exercise Training Workout Logs

Upon completing an exercise session the Workout Log will have to be completed. Each session requires you to:

1. Enter the number of minutes exercising and average heart rate recorded from the Polar FS1 Heart Rate Monitor
   - Information needs to be recorded immediately following your exercise session. Your exercise information is in the memory of the wrist, but will not be stored long-term. Exercise information is removed upon beginning a new session. FILE is protected for accidental start and information is saved only if the exercise recording has been on for more than one minute.

   1. After you finish your 30 minutes of exercising, press the front button on the watch. “STOP” will appear
   2. In the Time of Day display, press the front button until FILE is displayed.
   3. Wait for three seconds to enter the File. Your total exercise duration is displayed. **Record this total exercise duration.**
   4. Press the front button. Your average heart rate of the exercise session is displayed. **Record this average heart rate.**

2. Enter the date and the number of the exercise week (i.e. first week is Week 1, second week of exercise is Week 2 etc.)

3. Check that all stretches have been completed according to the stretches prescribed.

4. Enter the number of minutes warming-up

5. Circle the appropriate equipment used during session

6. Enter the number of minutes cooling-down

7. Add any additional comments regarding the exercise session, including muscle soreness, related injuries, and any barriers to completing a session.
## SEPT 2007

<table>
<thead>
<tr>
<th></th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>09/03/07</td>
<td>09/04/07</td>
<td>09/05/07</td>
<td>09/06/07</td>
<td>09/07/07</td>
<td>09/08/07</td>
<td>09/09/07</td>
</tr>
<tr>
<td>WEEK NUMBER</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Warm-up</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
</tr>
<tr>
<td>Exercise</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
<td>Minutes: 30</td>
</tr>
<tr>
<td>Avg HR</td>
<td>135</td>
<td>135</td>
<td>134</td>
<td>135</td>
<td>133</td>
<td>133</td>
<td>134</td>
</tr>
<tr>
<td>Equipment (circle one):</td>
<td>Treadmill</td>
<td>Treadmill</td>
<td>Treadmill</td>
<td>Treadmill</td>
<td>Treadmill</td>
<td>Treadmill</td>
<td>Treadmill</td>
</tr>
<tr>
<td></td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
<td>Elliptical Stepper</td>
</tr>
<tr>
<td>Cool-down</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
<td>Minutes: 5</td>
</tr>
<tr>
<td>Stretching</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Comments:
- Workout week starts on Thursday, February 9 (day 11 of cycle 2)
- Had muscle soreness on Friday, took Saturday off
- Had Wednesday off because completed five workouts in week 1
Scheduling Appointments and Questions

- Contact study coordinator, ALMA at (w) 612.968.9589 or (e) wiser@umn.edu to schedule Clinic Visits 1-6 and urine and food record pick-ups during menstrual cycles 2 and 6.

- Contact ANDREA at (w) 612.624.3050 or (e) plat0072@umn.edu with questions regarding food records and food frequency questionnaire.

- Contact BETH at (w) 612.626.8044, (c) 612.670.2702 or (e) moen0177@umn.edu with questions regarding the fitness assessments and exercise sessions.

- Contact AMANDA at (w) 612.626.8044, (c) 320.333.5460 or (e) thie0170@umn.edu with questions regarding the fitness assessments and exercise sessions.

- Contact LAURA at (w) 612.625.8693 or (e) rich0649@umn.edu if unable to reach other contacts.

For all contacts if leaving a message, give:
Your name and contact information, along with what early morning times you are available for clinic visit, exercise session, or urine collection (try to give a range of times, and what would be best. For example, “I am free 8-10, but would prefer 8:30.”)

PLEASE NOTE: If at any time you have questions or problems arise, please contact any of the WISER staff immediately. The WISER staff members are here to assist you through the study process, do not hesitate to call for any reason. The website may also provide you with study information and details: www.wiserwomen.umn.edu.

If we do not hear from you:
PLEASE NOTE: If a participant does not set up clinic appointments and/or attend exercise sessions when they would be expected to occur, a WISER staff member will make three (3) attempts to contact each participant, at various times, via phone and email, over 10 business days, advising the participant inquiring of her interest in continuing participation and advising of the possibility of termination of her study participation. The third message will state that the participant has 72 hours to contact WISER staff to set up rescheduling or to communicate that the participant has chosen to end her participation.
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Jaki0003@umn.edu
Map to GCRC (http://www.gGCRC.umn.edu/print/gGCRC/location/home.html): includes location, transportation, and parking information

Location
The GCRC is located on the 2nd floor of the Masonic and Veterans of Foreign Wars Building. This building is located on the East Bank of the University of Minnesota campus, adjacent to Fairview-University Medical Center. (It is shown as the Masonic Memorial Hospital on the map above, but the sign upon entering the building reads, “Masonic Cancer Center.”)

Phone: 612.626.0476
Fax: 612.626.2456
Mailing Address: MMC 504
420 Delaware St SE
Minneapolis, MN 55455
Shipping Address: 424 Harvard St SE
Masonic 211B – GCRC
Minneapolis, MN 55455

Public Transportation
Numerous city buses go to the Minneapolis campus via Washington Avenue, including 2, 16, 35A, 47D, 50, 52A, 52B, 52C, 52E, 52F, 52H, 52K, 52L, 52M, 52P, 52U, 95E, and 95U. Information on specific routes, times, and fares may be obtained by calling the Metro transit office at 612-373-3333 or going to their website at www.metrotransit.org.
Directions to GCRC from I-35W
These directions will take you to the Hospital Parking Ramp. Exit University Avenue and go east. Make a right (south) on Oak Street. Go past Washington Avenue (stop light) and make a right (west) on Delaware. Go 1½ blocks and enter parking ramp on right.

Directions to GCRC from I-94
These directions will take you to the Hospital Parking Ramp. Exit Huron Blvd go north. Go 2 blocks north to Washington Ave SE. Make a left (west). Go 2 blocks to Oak Street. Make a left (south) onto Oak Street. At the next intersection (Delaware Street) make a right (west) and go 1½ blocks until you get to the ramp.

Parking near GCRC
If you choose to drive to the GCRC, you have several choices for parking near the Clinical Research Center (GCRC) depending on the time of your appointment.

(1) The Hospital Parking Ramp is the closest to the GCRC. It is located on Delaware Avenue between Harvard and Walnut Streets. The ramp charges an hourly rate; you receive a ticket on the way into the ramp and pay on the way out. You will be charged a reduced rate ($2.50 for the first 30-60 minutes, $3.75 for 1-1.5 hours, $4.50 for 1.5-2.5 hours, and 2.5-3.5 hours) if you take your ticket to the GCRC and have it stamped.

(2) The Oak Street Ramp is a bit farther away, located off Oak Street. This is a good ramp to use if you have a 6:00, 6:30, or 7:00 a.m. appointment. Enter off Oak or Ontario streets and take a ticket as you enter. You will be charged $2.25 per hour based on the total time stamped on your ticket.

(3) The Washington Avenue ramp is located on Washington Avenue, next to the Radisson Hotel. This is also a good ramp to use for early morning appointments. To enter the ramp, turn right on Harvard St from Washington Avenue, then circle behind the ramp and enter on Union Street (this route is necessary due to one-way streets). You will be charged $2.25 per hour based on the total time stamped on your ticket.

(4) There is also metered parking available along Washington Avenue in front of the Radisson Hotel, off Delaware Street at Walnut, and off Harvard Street south of the GCRC. Meters are usually $1.25 per hour; please check individual metered areas for rates and times the meters are enforced.
Minneapolis YWCA Locations (http://www.ywcampls.org/locations/index.asp)

Downtown YWCA
Address
1130 Nicollet Mall
Minneapolis, MN 5540.
612.332.0501

Hours
Day        Time
Monday-Friday  6:00 am – 9:00 pm
Saturday     7:30 am – 5:00 pm
Sunday      9:00 am – 3:00 pm

Pay parking is available in the lot behind the building. The rate is $1 for 2 hours while you are in the facility. Enter from 12th Street between LaSalle Ave and Nicollet Mall.

Midtown YWCA
Address
2121 East Lake Street
Minneapolis, MN 55407
612.215.4333

Hours
Day        Time
Monday-Friday  5:30 am – 11:00 pm
Saturday & Sunday  7:30 am – 9:00 pm

The Midtown YWCA is steps from the Hiawatha Light Rail Station, is on several bus lines, and offers free parking in the adjacent lot.

Uptown YWCA
Address
2808 Hennepin Avenue South
Minneapolis, MN 55408
612.874.7131

Hours
Day        Time
Monday-Friday  5:30 am – 11:00 pm
Saturday & Sunday  7:30 am – 9:00 pm
Pay parking is available in the adjacent ramp. The rate is $1 for 2 hours while you are in our facility. Enter from Hennepin Avenue.

University of Minnesota Recreation Centers

University Recreation Complex
1906 University Avenue SE
Minneapolis, MN 55455
Map to University Recreation Complex

Public Transportation
Numerous city buses go to the Minneapolis campus via Washington Avenue, including 2, 16, 35A, 47D, 50, 52A, 52B, 52C, 52E, 52F, 52H, 52K, 52L, 52M, 52P, 52U, 95E, and 95U. Information on specific routes, times, and fares may be obtained by calling the Metro transit office at 612-373-3333 or going to their website at www.metrotransit.org.

Parking
(1) The 4th Street Ramp is located on the northeast corner of 4th Street SE and 17th Avenue SE. To enter the ramp turn right (north) off of 4th Street SE onto 17th Avenue SE and turn left to enter ramp. You will be charge $2.50 per hour for the first two hours and slightly less per hour for greater than two hours up to $12.00 maximum.

(2) The Washington Ave Ramp is located on Washington Avenue, next to the Radisson Hotel. To enter the ramp, turn right on Harvard St from Washington Avenue, then circle behind the ramp and enter on Union Street (this route is necessary due to one-way streets). However, if you exit after 8:00, you will be charged $1.75 per hour based on the total time stamped on your ticket.

(3) The Huron Blvd Parking Complex is located northeast of 4th Street SE and Oak Street SE. To enter the Huron Parking Complex travel east on University to Huron Blvd. North on Huron Blvd, or follow Huron Blvd North, or enter from south side (Huron Blvd/4th
St) or from north side (5th St). You will be charged $3.50 to park in the Huron Blvd Parking Complex.

### Hours

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday-Friday</td>
<td>5:45am-8:00pm</td>
</tr>
<tr>
<td>Saturday</td>
<td>10:00am-4:00pm</td>
</tr>
<tr>
<td>Sunday</td>
<td>12:00pm-5:00pm</td>
</tr>
</tbody>
</table>

**St. Paul Gymnasium**

1536 N Cleveland Avenue
St. Paul, MN 55108

[Map to St. Paul Gymnasium](#)

### Public Transportation

City buses going to the St. Paul campus include the 3 and 87. Information on specific routes, times, and fares may be obtained by calling the Metro transit office at 612-373-3333 or going to their website at [www.metrotransit.org](http://www.metrotransit.org).

Additionally, the Campus Connector provides direct bus service between the St. Paul, East Bank, and West Bank campuses approximately every five to 30 minutes from 7 a.m. through midnight, depending on the time of day and academic year.

### Parking

(1) There are Lot 171 Meters directly in front of the St. Paul Gym off of Cleveland Avenue N. Meters are $1.25 per hour.
(2) The Gortner Avenue Ramp is in between (south of) Buford Avenue and (north of) Commonwealth Avenue off of Gortner Avenue. The ramp can be entered off Gortner Avenue. You will be charged $2.50 per hour for the first two hours and slightly less per hour for greater than two hours up to $12.00 maximum.

(3) There is a limited amount of free one-hour street parking west of Cleveland Avenue near the St. Paul Gym.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday-Thursday</td>
<td>5:45am-8:00pm</td>
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<tr>
<td>Saturday</td>
<td>10:00am-4:00pm</td>
</tr>
<tr>
<td>Sunday</td>
<td>12:00pm-5:00pm</td>
</tr>
</tbody>
</table>
Data Collection Forms
# WISER (Women In Steady Exercise Research)

**Daily Food Record**

<table>
<thead>
<tr>
<th>Name: ____________________</th>
<th>Date: ________</th>
<th>Day: Su M T W Th F Sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Period: Baseline</td>
<td>Follow-up</td>
<td>Cycle Day: 7 8 9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Food or Beverage Consumed</th>
<th>Amount</th>
<th>Description/Preparation Method</th>
</tr>
</thead>
<tbody>
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_____ Typical day

_____ Not a typical day (please explain why):
## WISER (Women In Steady Exercise Research)

### Daily Food Record

<table>
<thead>
<tr>
<th>Name: _____________________</th>
<th>Date: __________</th>
<th>Day: Su M T W Th F Sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Period: Baseline Follow-up</td>
<td>Cycle Day: 7 8 9</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
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<th>Description/Preparation Method</th>
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______  Typical day

______  Not a typical day (please explain why):
WISER (Women In Steady Exercise Research)  
Daily Food Record

Name: _____________________ Date: ________ Day: Su M T W Th F Sa
Diet Period: Baseline Follow-up Cycle Day: 7 8 9

<table>
<thead>
<tr>
<th>Time</th>
<th>Food or Beverage Consumed</th>
<th>Amount</th>
<th>Description/Preparation Method</th>
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</thead>
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</tbody>
</table>

_____ Typical day  
_____ Not a typical day (please explain why):
Sleep Journal

Person ID

Menstrual cycle Day: 8  Date: _____ / ____ / ____

A. Complete these questions first thing in the morning.

1. At what time did you go to bed and try to fall asleep last night? ___ : ___  □ am □ pm

2. Did you wake up during the night?  □ No (0)  □ Yes (1)
   ⇒ If yes, at what time(s)?
   ___ : __  □ am □ pm
   ___ : __  □ am □ pm
   ___ : __  □ am □ pm

3. Did you sleep with any lights on?
   □ No (0)  □ Yes (1)   ⇒ If yes, what kind of light? _________
   For how long was the light on? _________

4. What time did you first wake up this morning?  ___ : ___  □ am □ pm

5. Did you go back to sleep after first waking up?
   □ No (0)  □ Yes (1)
   ⇒ If yes, what time did you get up and out of bed?  ___ : ___  □ am □ pm

6. Did you take any naps yesterday?  □ No (0)  □ Yes (1)  ⇒ If yes, at what times?

   Nap Begin  Nap End
   ___ : ___  □ am □ pm  ___ : ___  □ am □ pm
   ___ : ___  □ am □ pm  ___ : ___  □ am □ pm

7. How would you rate your sleep quality last night?

   1  2  3  4  5
   Very Poor  Below Average  Average  Above Average  Excellent
**Sleep Journal**

**Person ID**

| Menstrual cycle Day: 8 | Date: ___ / ___ / ___ |

---

**A. Complete these questions first thing in the morning.**

1. At what time did you go to bed and try to fall asleep last night?
   - __:__
   - am
   - pm

2. Did you wake up during the night?
   - No (0)
   - Yes (1)
   - If yes, at what time(s)?

3. Did you sleep with any lights on?
   - No (0)
   - Yes (1)
   - If yes, what kind of light? ______
   - For how long was the light on? ______

4. What time did you first wake up this morning?
   - __:__
   - am
   - pm

5. Did you go back to sleep after first waking up?
   - No (0)
   - Yes (1)
   - If yes, what time did you get up and out of bed?

6. Did you take any naps yesterday?
   - No (0)
   - Yes (1)
   - If yes, at what times?

<table>
<thead>
<tr>
<th>Nap Begin</th>
<th>Nap End</th>
</tr>
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<tbody>
<tr>
<td><strong>:</strong></td>
<td>am pm</td>
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<tr>
<td><strong>:</strong></td>
<td>am pm</td>
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<tr>
<td><strong>:</strong></td>
<td>am pm</td>
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</tbody>
</table>

7. How would you rate your sleep quality last night?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Poor</td>
<td>Below Average</td>
<td>Average</td>
<td>Above Average</td>
<td>Excellent</td>
</tr>
</tbody>
</table>
Sleep Journal

Person ID

Menstrual cycle Day: 8  Date: ____ / ____ / ___

A. Complete these questions first thing in the morning.

1. At what time did you go to bed and try to fall asleep last night? ___ : ___  □ am □ pm

2. Did you wake up during the night? □ No (0)  □ Yes (1)  ⇒ If yes, at what time(s)?
   ___ : ___  □ am □ pm
   ___ : ___  □ am □ pm
   ___ : ___  □ am □ pm

3. Did you sleep with any lights on? □ No (0)  □ Yes (1)  ⇒ If yes, what kind of light? _________
   For how long was the light on? _________

4. What time did you first wake up this morning? ___ : ___  □ am □ pm

5. Did you go back to sleep after first waking up? □ No (0)  □ Yes (1)  ⇒ If yes, what time did you get up and out of bed? ___ : ___  □ am □ pm

6. Did you take any naps yesterday? □ No (0)  □ Yes (1)  ⇒ If yes, at what times?

<table>
<thead>
<tr>
<th>Nap Begin</th>
<th>Nap End</th>
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<tbody>
<tr>
<td>___ : ___</td>
<td>□ am □ pm</td>
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<td>□ am □ pm</td>
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<tr>
<td>___ : ___</td>
<td>□ am □ pm</td>
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</tbody>
</table>

7. How would you rate your sleep quality last night?

1  2  3  4  5
Very Poor  Below Average  Average  Above Average  Excellent
## WORKOUT LOG

<table>
<thead>
<tr>
<th>Day</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
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### Week Number

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</table>

### Target Heart Rate

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<th>Avg HR:</th>
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<th>Avg HR:</th>
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### Warm Up

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### Exercise

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<tr>
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<th>Avg HR:</th>
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<th>Avg HR:</th>
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</table>

### Equipment (circle one):

- Treadmill
- Elliptical Stepper

### Cool-down

<table>
<thead>
<tr>
<th>Day</th>
<th>Minutes:</th>
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### Stretching

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### Comments

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## WORKOUT LOG

**NAME:** ____________________________  **STUDY ID:** ____________________________

### 2008

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Cool-down

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Week Number

Target Heart Rate

Warm Up

Exercise

Avg HR:

Equipment (circle one):

Stretching

Comments
## WORKOUT LOG

**NAME:** _______________________________  **STUDY ID:** _______________________________

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</table>
APPENDIX C-1

SAS CODE: ENDOGENOUS SEX HORMONES AND SHBG
/*Purpose of this code: create demographic variables*/

Data WISER.Demographics_319;
INFILE 'D:\Users\Alma\Desktop\School\SexHormones\Datasets\319\Demographics_319.csv' firstobs=2 DLM ="," DSD missover;
INPUT ID :$4.
   Group :$1.
   Entry_date :MMDDYY10.
   DOB :MMDDYY10.
      Ht_CV1 6.2
   Wt_CV1 6.2
   AmInALN :$1.
   Asian :$1.
      Black $1.
   Hawaiian :$1.
      White $1.
      OthRH $1.
   Hispanic $3.
   EDU :$40.
   BCuse :$1.
   Parity :1.
;
FORMAT ID $4.
   Group $1.
   Entry_date MMDDYY10.
   DOB MMDDYY10.
      Ht_CV1 6.2
   Wt_CV1 6.2
   AmInALN $1.
   Asian $1.
      Black $1.
   Hawaiian $1.
      White $1.
      OthRH $1.
   Hispanic $3.
   EDU $40.
      MarStat $18.
   BCuse $1.
   Parity 1.
;
RUN;

Data WISER.Demographics_319;
SET WISER.Demographics_319;

   length trt $8.;
   if group=1 then trt='exercise';
   if group=2 then trt='control';

   Age=((Entry_Date-DOB)/365.25);
   length Age_strata $9.;
If Age LT 25 then Age_Strata = '1_under25';
If Age GE 25 then Age_Strata = '2_over25';

BMI = (Wt_CV1)/((Ht_CV1/100)**2);
/*BMI=body weight in kg/ height in m squared*/

length BMI_strata $9.;
If BMI LT 22.8 then BMI_Strata = '1_lowBMI';
If BMI GE 22.8 and BMI LT 26.3 then BMI_Strata = '2_midBMI';
If BMI GE 26.3 then BMI_Strata = '3_highBMI';

length BMI_class $8.;
If BMI GE 0 and BMI LT 25.0 then BMI_Class = '1_Normal';
If BMI GE 25.0 and BMI LT 30.0 then BMI_Class = '2_Overwt';
If BMI GE 30.0 then BMI_Class = '3_Obese';

length BMI_class4 $9.;
BMI_class4 = BMI_class;
If BMI GE 0 and BMI LT 18.5 then BMI_Class4 = '0_Underwt';

length BMI_over25 $8.;
if BMI GE 18.5 and BMI LT 25.0 then BMI_over25 = '1_Normal';
if BMI GE 25.0 then BMI_over25 = '2_over25';

If EDU = 'College/University degree' then
  Education_cat = 'College or more';
If EDU = 'Graduate or Professional degree' then Education_cat = 'College or more';
If EDU = 'Some college (less than 4 years)' then Education_cat = 'Some college';
If EDU = 'Vocational training (beyond high school)' then Education_cat = 'HS or less';
If EDU = 'Completed high school diploma (or GED)' then Education_cat = 'HS or less';
IF EDU = 'Less than high school diploma' then
  Education_cat = 'HS or less';

If White = '1' then race = 'White';
else if Black = '1' then race = 'Black';
else if Asian = '1' then race = 'Asian';
else race = 'Other';

If marstat = 'Divorced' then status = 'not together';
if marstat = 'Separated' then status = 'not together';
if marstat = 'Married' then status = 'together';
if marstat = 'Domestic partnered' then status = 'together';
if marstat = 'Never married' then status = 'never';

FORMAT Age 5.1
  BMI 5.1;
RUN;

="/**************************
Purpose of this code: to create body composition variables
ID 1960 and 2663 did not complete follow up DEXA
0 baseline, 1 follow up
***************************************************************************/

202
DATA WISER.Bodycomp_319;
INFILE 'D:\Users\Alma\Desktop\School\SexHormones\Datasets\319\Bodycomp_319.csv' firstobs=2 DLM = " DSD missover;
INPUT ID :$4. ToTissuepre :6.0 ToFatpre :5.0
   ToLeanpre :5.0 ToTissuefu :6.0 ToFatfu :5.0
   ToLeanfu :5.0;
FORMAT ID $4. ToTissuepre 6.0
   ToFatpre 5.0
   ToLeanpre 5.0
   ToTissuefu 6.0
   ToFatfu 5.0
   ToLeanfu 5.0;
RUN;

DATA WISER.Bodycomp_319;
set WISER.Bodycomp_319;

Fat_mass_0= ToFatpre/1000.0;
Fat_mass_1= ToFatfu/1000.0;
Percent_bodyfat_0=(ToFatpre/ToTissuepre)*100;
Percent_bodyfat_1=(ToFatfu/ToTissuefu)*100;
Lean_mass_0= ToLeanpre/1000.0;
Lean_mass_1= ToLeanfu/1000.0;
Percent_leanmass_0=(ToLeanpre/ToTissuepre)*100;
Percent_leanmass_1=(ToLeanfu/ToTissuefu)*100;

array a[4] fat_mass_0 lean_mass_0 percent_bodyfat_0 percent_leanmass_0;
array b[4] fat_mass_1 lean_mass_1 percent_bodyfat_1 percent_leanmass_1;
array c[4] D_fat_mass D_lean_mass D_percent_bodyfat D_percent_leanmass;
array d[4] PD_fat_mass PD_lean_mass PD_percent_bodyfat PD_percent_leanmass;

do j=1 to 4;
c[j]= b[j] - a[j];
d[j]= (100.0* c[j])/a[j];
end;

FORMAT Fat_mass_0 5.3
   Fat_mass_1 5.3
   D_fat_mass 5.2
   PD_fat_mass 5.2
   Percent_bodyfat_0 5.2
   Percent_bodyfat_1 5.2
   D_percent_bodyfat 5.2
   PD_percent_bodyfat 5.2
   Lean_mass_0 5.2
   Lean_mass_1 5.2
   D_lean_mass 5.2
   PD_lean_mass 5.2
   Percent_leanmass_0 5.2
PROC PRINT data=WISER.Bodycomp_319 (OBS=5);
RUN;
Data WISER.Menarche_310;
INFILE 'D:\Users\Alma\Desktop\School\SexHormones\Datasets\319\Menarche_310.csv'
firstobs=2 DLM ="," DSD missover;
INPUT ID         :$4.
   F25q1      :4.1
;
FORMAT ID $4.
   F25q1  4.1
;
RUN;
Data WISER.Menarche_310 (rename=(F25q1=Menarche_age));
SET WISER.Menarche_310;
RUN;
Data WISER.FamilyHistory_251;
INFILE 'D:\Users\Alma\Desktop\School\SexHormones\Datasets\319\BC_251.csv'
firstobs=2 DLM ="," DSD missover;
   First_degree_relatives :1.
   Second_degree_relatives :1.;
FORMAT ID $4.
   Group $1.
   First_degree_relatives 1.
   Second_degree_relatives 1.;
RUN;
Data WISER.FamilyHistory_251;
Set wiser.FamilyHistory_251;
length BC_history $3;
if First_degree_relations = 0 then BC_history = 'NO';
else BC_history = 'YES';
drop group;
run;

Data WISER.Foodrecords_312_0;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\Foodrecords_312_0.csv';
firstobs=2 DLM = "", DSD missover LRECL=386;

INPUT ID:$4. timepoint :$1. Kcal:7.2 FatCals:7.2 SatCals:6.2 Prot_g:6.2 Carb_g:7.2 Fib_g:6.2 SolFib_g:5.2 Sugar_g:7.2 MonSac_g:6.2 Disacc_g:6.2 Ocarb_g:7.2 Fat_g:6.2 SatFat_g:6.2 MonoFat_g:5.2 PolyFat_g:5.2 TransFat_g:5.2 Chol_mg:7.2 Water_g:8.2 vitA_IU:8.2 vitA_RAE:7.2 Carotene_RE:7.2 etinol_RE:7.2 Bcarot_mcg:7.2 B1_mg:5.2 B2_mg:5.2 B3_mg:6.2 B3_NE_mg:6.2 B6_mg:5.2 B12_mcg:5.2 Biot_mcg:5.2 VitC_mg:7.2 VitK_mcg:6.2 Panto_mg:6.2 Calcium_mg:7.2 Chrom_mcg:5.2 Copp_mg:5.2 Fluor_mg:5.2 Iodine_mcg:6.2 Iron_mg:6.2 Magn_mg:7.2 Mang_mg:5.2 Moly_mcg:5.2 Phos_mg:7.2 Pot_mg:8.2 Sel_mcg:6.2 Sod_mg:8.2 Zinc_mg:5.2 Omeg3_g:5.2 Omeg6_g:6.2 Wgt_g:8.2 Alc_g:7.2 Caff_mg:7.2 Chln_mg:7.2;
RUN;

Data wiser.Foodrecords_312_0;
Set wiser.Foodrecords_312_0(rename=(Kcal=kcal_0 FatCals=Fatcals_0 SatCals=satcals_0 Prot_g=Prot_g_0 Carb_g=Carb_g_0 Fib_g=Fib_g_0 SolFib_g=SolFib_g_0 Sugar_g=Sugar_g_0 MonSac_g=MonSac_g_0 Disacc_g=Disacc_g_0 Ocarb_g=Ocarb_g_0 Fat_g=Fat_g_0 SatFat_g=SatFat_g_0 MonoFat_g=MonoFat_g_0 PolyFat_g=PolyFat_g_0 TransFat_g=TransFat_g_0 Chol_mg=Chol_mg_0 Water_g=Water_g_0 B1_mg=B1_mg_0 B2_mg=B2_mg_0 B3_mg=B3_mg_0 B3_NE_mg=B3_NE_mg_0 B6_mg=B6_mg_0 B12_mcg=B12_mcg_0 Biot_mcg=Biot_mcg_0 VitC_mg=VitC_mg_0 VitD_1U=vitD_1U_0 vitD_mcg=vitD_mcg_0 tocopherol_mg=tocopherol_mg_0 Folate_mcg=Folate_mcg_0 Fol_DFE_mcg=Fol_DFE_mcg_0 VitK_mcg=VitK_mcg_0 Panto_mg=Panto_mg_0 Calcium_mg=Calcium_mg_0 Chrom_mcg=Chrom_mcg_0 Copp_mg=Copp_mg_0 Fluor_mg=Fluor_mg_0 Iodine_mcg=Iodine_mcg_0 Iron_mg=Iron_mg_0 Magn_mg=Magn_mg_0 Mang_mg=Mang_mg_0 Moly_mcg=Moly_mcg_0 Phos_mg=Phos_mg_0 Pot_mg=Pot_mg_0 Sel_mcg=Sel_mcg_0 Sod_mg=Sod_mg_0 Zinc_mg=Zinc_mg_0 Omeg3_g=Omeg3_g_0 Omeg6_g=Omeg6_g_0 Wgt_g=Wgt_g_0 Alc_g=Alc_g_0 Caff_mg=Caff_mg_0 Chln_mg=Chln_mg_0));
RUN;

Data WISER.Foodrecords_312_1;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\Foodrecords_312_1.csv';
firstobs=2 DLM = "", DSD missover LRECL=386;

SatCals: 6.2 Prot_g: 6.2 Carb_g: 7.2 Fib_g: 6.2 SolFib_g: 5.2 Sugar_g: 7.2 MonSac_g: 6.2
Disacc_g: 6.2 Ocarb_g: 7.2 Fat_g: 6.2 SatFat_g: 6.2 MonoFat_g: 5.2 PolyFat_g: 5.2
TransFat_g: 5.2 Chol_mg: 7.2 Water_g: 8.2 vitA_IU: 8.2 vitA_RAE: 7.2 Carotene_RE: 7.2
etinol_RE: 7.2 Bcarot_mcg: 7.2 B1_mg: 5.2 B2_mg: 5.2 B3_ne: 6.2 B9_mg: 5.2 B12_mcg: 5.2
Biot_mcg: 5.2 VitC_mg: 6.2 VitD_IU: 7.2 vitD_mcg: 5.2 tocopherol_mg: 6.2 Folate_mcg: 7.2 Fol_DFE_mcg: 7.2
VitK_mcg: 6.2 Panto_mg: 6.2 Calcium_mg: 7.2 Chrom_mg: 5.2 Copp_mg: 5.2
Fluor_mg: 5.2 Iodine_mcg: 6.2 Iron_mg: 6.2 Magn_mg: 7.2 Mang_mg: 5.2 Moly_mcg: 5.2 Phos_mg: 7.2 Pot_mg: 8.2 Sel_mcg: 6.2 Sod_mg: 8.2
Zinc_mg: 5.2 Omeg3_g: 5.2 Omeg6_g: 6.2 Wgt_g: 8.2 Alc_g: 7.2 Caff_mg: 7.2 Chln_mg: 7.2;

RUN;

Data wiser.Foodrecords_312_1;
Set wiser.Foodrecords_312_1(rename=(Kcal=kcal_1 FatCals=Fatcals_1
SatCals=sateals_1 Prot_g= Prot_g_1 Carb_g=Carb_g_1 Fib_g=Fib_g_1 SolFib_g=SolFib_g_1
Sugar_g=Sugar_g_1 MonSac_g=MonSac_g_1 Disacc_g=Disacc_g_1 Ocarb_g=Ocarb_g_1
Fat_g=Fat_g_1 SatFat_g=SatFat_g_1
 MonoFat_g=MonoFat_g_1 PolyFat_g=PolyFat_g_1 TransFat_g=TransFat_g_1
Chol_mg=Chol_mg_1 Water_g=Water_g_1 B1_mg=B1_mg_1
B2_mg=B2_mg_1 B3_mg=B3_mg_1 B9_mg=B9_mg_1 B12_mg=B12_mg_1
B12_mcg=B12_mcg_1 Biot_mcg=Biot_mcg_1 VitC_mg=VitC_mg_1
vitD_IU=VitD_IU_1 vitD_mcg=VitD_mcg_1 tocopherol_mg=tocopherol_mg_1
Folate_mcg=Folate_mcg_1 Fol_DFE_mcg=Fol_DFE_mcg_1
VitK_mcg=VitK_mcg_1 Panto_mg=Panto_mg_1 Calcium_mg=Calcium_mg_1
Chrom_mg=Chrom_mg_1 Copp_mg=Copp_mg_1 Fluor_mg=Fluor_mg_1
Iodine_mcg=Iodine_mcg_1 Iron_mg=Iron_mg_1 Magn_mg=Magn_mg_1
Mang_mg=Mang_mg_1 Moly_mcg=Moly_mcg_1 Phos_mg=Phos_mg_1
Pot_mg=Pot_mg_1 Sel_mcg=Sel_mcg_1 Sod_mg=Sod_mg_1 Zinc_mg=Zinc_mg_1
Omeg3_g=Omeg3_g_1 Omeg6_g=Omeg6_g_1 Wgt_g=Wgt_g_1
Alc_g=Alc_g_1 Caff_mg=Caff_mg_1 Chln_mg=Chln_mg_1));

run;

Data wiser.Foodrecords_312;
Merge wiser.Foodrecords_312_0 wiser.Foodrecords_312_1;
by ID;
drop timepoint;
run;

Data wiser.Foodrecords_312;
set wiser.Foodrecords_312;
D_kcal=kcal_1 - kcal_0;
PD_kcal = 100.0*D_kcal/kcal_0;
run;

options ls=100 nodate pageno=1 NOFMTERR;
options mprint;
* wiser01.sas 26 Feb 2010 ;
* analysis data sets:

206
wiser.exercise_compliance  (n = 188) from exercise logs

wiser adherence (exercise adherence, merged back into strata in wiser00)

self-reported physical activity:
NOTE: There were 402 observations read from the data set WORK.SRPA_0. (baseline)
NOTE: There were 319 observations read from the data set WORK.SRPA_1. (final)
NOTE: There were 128 observations read from the data set WORK.SRPA_2. (final+ 6 months)
NOTE: The data set WISER.SRPA has 403 observations and 10 variables;

*************************************************** ****************;
* macro to read job and leisure datasets, match leisure to activity code, and calculate METS per week;
*************************************************** ****************;

%macro activity (job=,leisure=,out=,ext=);
proc sort data = &leisure;
* mult obs per id;
by activity_code;
proc sort data = wiser.paicomptable;
by activity_code;
proc sort data=&job;
by id;

Occupational categories:
if category = 0 then job_mets = 0
if category = 1 then job_mets = 2
if category = 1.5 then job_mets = 3
if category = 2 then job_mets = 4
if category = 2.5 then job_mets = 5
if category = 3 then job_mets = 6;

proc contents data= &leisure;
proc contents data= wiser.paicomptable;
proc contents data= &job;

data leisure1;
merge &leisure wiser.paicomptable;
by activity_code;
if id=. then delete;
if (activity_name="treading water") then mets=6;
sum_months=sum(jan,feb,mar,apr,may,jun,jul,aug,sep,oct,nov,dec);
leisure_mets = mets*(sum_months * Avg___times * Avg___min)/(60.0 * 52.0);
keep id activity_code leisure_mets;

data job1;
set &job ;
if id NE . ;
if (__hrs_day < __hrs_sitting) then do;
  if (id=1068) then __hrs_sitting = __hrs_sitting/__Days_week;
  else delete;
end;
job_act_hrs = __hrs_day - __hrs_sitting + ( Walk_bike_min/60.0);
job_mets = 2.0*category*__Months * __Days_week * job_act_hrs /52.0;
keep id job_name job_mets;
proc sort data=leisure1; by id;
proc sort data=job1; by id;
proc print data=leisure1 (obs=20);
proc print data=job1 (obs=20);
data mets0;
set leisure1 job1;
proc sort data=mets0;
bysid;
data &out;
set mets0;
bysid;
if (first.id=1) then do;
total_mets_week&ext=0; leisure_mets_week&ext=0; job_mets_week&ext=0;
end;
retain total_mets_week&ext leisure_mets_week&ext job_mets_week&ext;
leisure_mets_week&ext = sum(leisure_mets, leisure_mets_week&ext);
job_mets_week&ext = sum(job_mets, job_mets_week&ext);
total_mets_week&ext = sum(leisure_mets,job_mets, total_mets_week&ext);
if (last.id=1) then output;
keep id total_mets_week&ext leisure_mets_week&ext job_mets_week&ext;
FORMAT ID$4.;
proc print data=&out(obs=20);
%mend activity;
*************************************************** ****************;
*************************************************** ****************;
%activity(job=wiser.paiocctable_pre, leisure=wiser.paiacctable_pre,out=srpa_0,ext=_0);
%activity(job=wiser.PAIOcctable_POST, leisure=wiser.PAIActable_POST,out=srpa_1,ext=_1);
/*/ %activity(job=wiser.PAI0ccctble_6mo_post, leisure=wiser.PAIActtbl_6mo_post,out=srpa_2,ext=_2); */
data wiser.srpa;
merge srpa_0 srpa_1 /*srpa_2*/;
bysid;
proc export data=wiser.srpa
outfile="D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\Original datasets\SRPA.xls"
dbms=excel replace;
run; quit;
/*
data check1;
set leisure;
if srmets < 0;
proc print;
data check2;
set job;
if srmets < 0;*/
proc print;

proc print data=wiser.srpa;
  where mets_week > 100.0;
proc print data=leisure;
  where (id=1841 or id=2316);
proc print data=job (obs=20);
  where (id=1841 or id=2316);
*/

Data wiser.SRPA;
Merge WISER.SRPA;
BY id;
D_SRPA= total_mets_week_1 - total_mets_week_0;
if (total_mets_week_0 >0) then D_percent_srpa = 100.0*D srpa/total_mets_week_0;
else D percent srpa = .;
run;

Data WISER.SRPA_319;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\SRPA_319.csv'
firstobs=2 DLM="\" DSD missover;
INPUT ID :$4.
  Total_mets_week_0  :5.2
  Leisure_mets_week_0 :5.2
  Job_mets_week_0     :5.2
  Total_mets_week_1   :5.2
  Leisure_mets_week_1 :5.2
  Job_mets_week_1     :5.2;
FORMAT ID     :$4.
  Total_mets_week_0  :5.2
  Leisure_mets_week_0 :5.2
  Job_mets_week_0     :5.2
  Total_mets_week_1   :5.2
  Leisure_mets_week_1 :5.2
  Job_mets_week_1     :5.2;
run;

Data wiser.SRPA_319;
Set WISER.SRPA_319;
D SRPA= total_mets_week_1 - total_mets_week_0;
if (total_mets_week_0 >0) then D_percent_srpa = 100.0*D srpa/total_mets_week_0;
else D percent srpa = .;
FORMAT D_SRPA     :6.2
  D_percent_srpa :7.2;
run;
PROC PRINT;
RUN;
/*Purpose of this code: Create fitness variables*/

DATA WISER.Fitness_319;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\Fitness_319.csv'
firstobs=2 DLM =",", DSD missover;
INPUT ID :$4. Group :$1. MET_BL :5.2 MET_FU :5.2;
FORMAT ID $4. Group $1. MET_BL 5.2 MET_FU 5.2;
RUN;

DATA WISER.Fitness_319 (rename=(MET_BL=Mets_0 MET_FU= Mets_1));
Set WISER.Fitness_319;
run;

DATA WISER.Fitness_319;
Set WISER.Fitness_319;
D_Mets= Mets_1-Mets_0;
PD_Mets= 100.0*D_Mets/Mets_0;
Format D_Mets 5.2
   PD_Mets 5.2;
RUN;

Data b;
Set WISER.Demographics_319;
keep id;
run;

Data a1;
Merge WISER.exercise_adherence (in=in_a) b(in=in_b);
by id;
from_a = in_a;
from_b = in_b;
run;

Data a2;
Set a1;
if (from_a NE from_b);
run;
proc print data=a2;
run;

Data a3;
set a1;
if from_a =1 and from_b =1;
run;
proc print data=a3;
run;

Data a4;
set a3;
keep id Exercise_complier;
run;

Data a5;
set wiser.bodyweights_319;
keep id weight_complier;
run;
proc print;
run;
Data a6;
Merge a3 a5;
drop from_a from_b;
run;

Data wiser.compliance;
set a6;
complier= (weight_complier=1 and exercise_complier=1);
run;
proc print data=wiser.compliance;
run;

/*PURPOSE OF THIS CODE: properly format WISER.Hormones_319.csv
Units for E2, BE2, FE2,FT = pg/ml
for TT, BT, and P=ng/ml
for SHBG = nmol/L*/

Data WISER.Hormones;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\Hormones_319.csv'
firstobs=2 DLM ="," DSD missover;

INPUT ID :S4.
   Group :S1.
      E2_0 :7.3
      E2_1 :7.3
          BE2_0 :7.3
          BE2_1 :7.3
             FE2_0 :6.3
             FE2_1 :6.3
              E1SO4_0 :6.3
              E1SO4_1 :6.3
       TT_0 :6.3
       TT_1 :6.3
        BT_0 :6.3
        BT_1 :6.3
FT_0 : 6.3
FT_1 : 6.3
SHBG_0 : 7.3
SHBG_1 : 7.3
P_0 : 6.3
P_1 : 6.3
;

FORMAT ID $4.
  Group $1.
  E2_0  7.3
  E2_1  7.3
  BE2_0  7.3
  BE2_1  7.3
  FE2_0  6.3
  FE2_1  6.3
  E1SO4_0  6.3
  E1SO4_1  6.3
  TT_0  6.3
  TT_1  6.3
  BT_0  6.3
  BT_1  6.3
  FT_0  6.3
  FT_1  6.3
  SHBG_0  7.3
  SHBG_1  7.3
  P_0  6.3
  P_1  6.3
;

if (ID=1045) then P_1=.; /*CV6 blood drawn on day 8 instead of 8 days after ovulation*/
drop group;
run;

Data wiser.change_hormones_319;
Set WISER.hormones;

Format
  D_E2  7.2
  D_BE2  6.2
  D_FE2  6.2
  D_E1SO4  6.2
  D_TT  6.2
  D_BT  6.2
  D_FT  6.2
  D_SHBG  6.2
  D_P  6.2
  PD_E2  6.2
  PD_BE2  7.2
  PD_FE2  7.2
  PD_E1SO4  7.2
  PD_TT  7.2
  PD_BT  7.2

212
array a[9] E2_0 BE2_0 FE2_0 E1SO4_0 TT_0 BT_0 FT_0 SHBG_0 P_0;
array b[9] E2_1 BE2_1 FE2_1 E1SO4_1 TT_1 BT_1 FT_1 SHBG_1 P_1;
array c[9] D_E2 D_BE2 D_FE2 D_E1SO4 D_TT D_BT D_FT D_SHBG D_P;

do j=1 to 9;
   c[j]= b[j]-a[j];
   d[j]= 100.0*c[j]/a[j];
end;
Drop j;
RUN;

Proc Means Data=wiser.change_hormones_319
nmiss max min mean stddev;
var E2_0 BE2_0 FE2_0 E1SO4_0 TT_0 BT_0 FT_0 SHBG_0 P_0;
run;

Proc Univariate plot Data=wiser.change_hormones_319;
var E2_0 BE2_0 FE2_0 E1SO4_0 TT_0 BT_0 FT_0 SHBG_0 P_0;
run;

Proc Means Data=wiser.change_hormones_319
nmiss max min mean median stddev;
var D_E2 D_BE2 D_FE2 D_E1SO4 D_TT D_BT D_FT D_SHBG D_P;
run;

Proc Univariate plot DATA=WISER.change_hormones_319;
var D_E2 D_BE2 D_FE2 D_E1SO4 D_TT D_BT D_FT D_SHBG D_P;
run;

/*All hormones are right-skewed (max/min >10) so need to log transform*/

Data wiser.log_hormones_319;
Set wiser.hormones;
log_E2_0     = log(E2_0) 
log_E2_1     = log(E2_1) 
log_BE2_0    = log(BE2_0) 
log_BE2_1    = log(BE2_1) 
log_FE2_0    = log(FE2_0) 
log_FE2_1    = log(FE2_1) 
log_E1SO4_0  = log(E1SO4_0)
log_E1SO4_1  = log(E1SO4_1)
log_TT_0     = log(TT_0) 
log_TT_1     = log(TT_1) 
log_BT_0     = log(BT_0) 
log_BT_1     = log(BT_1) 
log_FT_0     = log(FT_0) 
log_FT_1     = log(FT_1) 
log_SHBG_0   = log(SHBG_0) ;
log_SHBG_1 = log(SHBG_1) ;
log_P_0 = log(P_0) ;
log_P_1 = log(P_1) ;

Format
log_E2_0 7.2
log_E2_1 7.2
log_BE2_0 7.2
log_BE2_1 7.2
log_FE2_0 7.2
log_FE2_1 7.2
log_E1SO4_0 7.2
log_E1SO4_1 7.2
log_TT_0 7.2
log_TT_1 7.2
log_BT_0 7.2
log_BT_1 7.2
log_FT_0 7.2
log_FT_1 7.2
log_SHBG_0 7.2
log_SHBG_1 7.2
log_P_0 7.2
log_P_1 7.2

keep ID log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 log_TT_0 log_BT_0 log_FT_0 log_SHBG_0
log_P_0 log_E2_1 log_BE2_1 log_FE2_1 log_E1SO4_1 log_TT_1 log_BT_1 log_FT_1 log_SHBG_1
log_P_1 ;
run;

Data WISER.Hormones_319;
Merge wiser.log_hormones_319 wiser.change_hormones_319;
drop E2_0 BE2_0 FE2_0 E1SO4_0 TT_0 BT_0 FT_0 SHBG_0 P_0 E2_1 BE2_1 FE2_1 E1SO4_1
TT_1 BT_1 FT_1 SHBG_1 P_1 ;
run;

/************************************************** ********
IDs 1960, 1996, 2021, 2030, 2062, and 2638 didn't do follow-up weight so use CV5 wt instead
ID 2663 didn't do CV6, CV5 or CV4 so use CV1 wt instead
*************************************************** *****/

Data ht;
Set WISER.demographics_319;
keep id ht_cv1;
run;

Data wiser.bodyweights_319;;
INFILE 'D:\Users\Alma\Desktop\School\Sex Hormones\Datasets\319\Bodyweights_319.csv'
firstobs=2 DLM ="," DSD missover;
INPUT ID :$4. WeightCV1 :5.1 WeightCV4 :5.1
WeightCV5 :5.1 WeightCV6 :5.1 ;
FORMAT
ID $4.
WeightCV1 5.1
WeightCV4 5.1
WeightCV5 5.1
WeightCV6 5.1;

RUN;
DATA wiser.bodyweights_319;
MERGE HT Wiser.bodyweights_319 (rename=(WeightCV1=weight_0 WeightCV4=weight_2months WeightCV5=weight_3months WeightCV6=weight_1));
D_weight= weight_1 - weight_0;
P_D_weight=100.0* D_weight/weight_0;
weight_complier = (-4.0 LE PD_weight LE 4.0);
BMI_0=(Weight_0)/(Ht_CV1/100.0)**2;
BMI_1=(Weight_1)/(Ht_CV1/100.0)**2;
D_BMI= BMI_1 - BMI_0;
P_D_BMI = 100.0* D_BMI/BMI_0;

FORMAT
BMI_0 4.1
BMI_1 4.1
D_BMI 5.2
PD_BMI 5.2;
RUN;

PROC FREQ DATA=WISER.Bodyweights_319;
TABLES weight_complier;
RUN;

OPTIONS LS=100 NODATE PAGENO=1 NOFMTERR;
OPTIONS MPRINT;

* wiser01.sas 26 Feb 2010 ;

* analysis data sets:
  wiser.exercise_compliance (n = 188) from exercise logs
  wiser.adherence (exercise adherence, merged back into strata in wiser00)

  self-reported physical activity:
  NOTE: There were 402 observations read from the data set WORK.SRPA_0. (baseline)
  NOTE: There were 319 observations read from the data set WORK.SRPA_1. (final)
  NOTE: There were 128 observations read from the data set WORK.SRPA_2. (final+ 6 months)
  NOTE: The data set WISER.SRPA has 403 observations and 10 variables;

DATA a;
  SET wiser.exercise_logs;
  IF (id NE .);
  IF (week NE .);
  IF (0 < stage_of_exercise LE 4);
proc sort data = a;
by id stage_of_exercise week day;

data wiser.exercise_compliance;
set a;
by id stage_of_exercise week day;
if (id = 1504 and week > 12) then stage_of_exercise=4; * missing;
if (id = 1641 and (5 LE week LE 9)) then stage_of_exercise=2; * missing;
if (id = 1641 and (10 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 1641 and (13 LE week LE 14)) then stage_of_exercise=4; * missing;
if (id = 1768 and (11 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 1768 and (13 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 1874 and (0 < week LE 5)) then stage_of_exercise=1; * missing;
if (id = 1985 and (17 LE week LE 18)) then stage_of_exercise=4; * missing;
if (id = 2120 and (10 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 2120 and (13 LE week LE 17)) then stage_of_exercise=4; * missing;
if (id = 2128 and THRlow=.) then delete; * missing;
if (id = 2128 and ( week = 6)) then stage_of_exercise=2; * missing;
if (id = 2156 and (0 LE week LE 3)) then stage_of_exercise=1; * missing;
if (id = 2156 and (4 LE week LE 8)) then stage_of_exercise=2; * missing;
if (id = 2156 and (9 LE week LE 10)) then stage_of_exercise=3; * missing;
if (id = 2156 and (11 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 2218 and ( week = 4)) then stage_of_exercise=1; * missing;
if (id = 2218 and (5 LE week LE 6)) then stage_of_exercise=2; * missing;
if (id = 2510) then delete; * missing target HR, stage of exercise;
if (id = 2555 and ( week = 12)) then stage_of_exercise=3; * missing;
if (id = 2559 and ( week = 16)) then stage_of_exercise=4; * missing;
if (id = 2563 and ( week = 1)) then stage_of_exercise=1; * missing;
if (id = 2564 and ( week = 10)) then stage_of_exercise=3; * missing;
if (id = 2564 and ( week = 13)) then stage_of_exercise=4; * missing;
if (id = 2579 and (6 LE week LE 8)) then stage_of_exercise=2; * missing;
if (id = 2579 and (9 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 2579 and (13 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 2656 and (9 LE week LE 11)) then stage_of_exercise=3; * missing;
if (id = 2679 and (6 LE week LE 7)) then stage_of_exercise=2; * missing;
if (id = 2689 and (5 LE week LE 6)) then stage_of_exercise=2; * missing;
if (id = 2689 and (9 LE week LE 10)) then stage_of_exercise=3; * missing;
if (id = 2691 and ( week = 8)) then stage_of_exercise=2; * missing;
if (id = 2691 and ( week = 9)) then stage_of_exercise=3; * missing;
array obs[4] obs1 - obs4; * minutes exercised at each exercise stage;
array exp[4] exp1 - exp4; * assigned days of exercise at each exercise stage;
array c[4] compl1-compl4; * percent minutes exercised/minutes assigned compliance at each exercise stage;
retain obs1 - obs4 exp1 - exp4 compl1-compl4 total_obs total_exp total_compl;
if (first.id=1) then do j = 1 to 4; obs[j] = 0; exp[j]=0; c[j]=0;
total_obs=0; total_exp=0; total_compl=0;
end;
total_obs = SUM(ExM, total_obs);
obs[stage_of_exercise] = SUM(ExM, obs[stage_of_exercise]);
if (last.week = 1) then do;
exp[stage_of_exercise] = SUM(exp[stage_of_exercise], Work_out_days_per_week);

proc sort data = a;
by id stage_of_exercise week day;

data wiser.exercise_compliance;
set a;
by id stage_of_exercise week day;
if (id = 1504 and week > 12) then stage_of_exercise=4; * missing;
if (id = 1641 and (5 LE week LE 9)) then stage_of_exercise=2; * missing;
if (id = 1641 and (10 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 1641 and (13 LE week LE 14)) then stage_of_exercise=4; * missing;
if (id = 1768 and (11 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 1768 and (13 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 1874 and (0 < week LE 5)) then stage_of_exercise=1; * missing;
if (id = 1985 and (17 LE week LE 18)) then stage_of_exercise=4; * missing;
if (id = 2120 and (10 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 2120 and (13 LE week LE 17)) then stage_of_exercise=4; * missing;
if (id = 2128 and THRlow=.) then delete; * missing;
if (id = 2128 and ( week = 6)) then stage_of_exercise=2; * missing;
if (id = 2156 and (0 LE week LE 3)) then stage_of_exercise=1; * missing;
if (id = 2156 and (4 LE week LE 8)) then stage_of_exercise=2; * missing;
if (id = 2156 and (9 LE week LE 10)) then stage_of_exercise=3; * missing;
if (id = 2156 and (11 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 2218 and ( week = 4)) then stage_of_exercise=1; * missing;
if (id = 2218 and (5 LE week LE 6)) then stage_of_exercise=2; * missing;
if (id = 2510) then delete; * missing target HR, stage of exercise;
if (id = 2555 and ( week = 12)) then stage_of_exercise=3; * missing;
if (id = 2559 and ( week = 16)) then stage_of_exercise=4; * missing;
if (id = 2563 and ( week = 1)) then stage_of_exercise=1; * missing;
if (id = 2564 and ( week = 10)) then stage_of_exercise=3; * missing;
if (id = 2564 and ( week = 13)) then stage_of_exercise=4; * missing;
if (id = 2579 and (6 LE week LE 8)) then stage_of_exercise=2; * missing;
if (id = 2579 and (9 LE week LE 12)) then stage_of_exercise=3; * missing;
if (id = 2579 and (13 LE week LE 15)) then stage_of_exercise=4; * missing;
if (id = 2656 and (9 LE week LE 11)) then stage_of_exercise=3; * missing;
if (id = 2679 and (6 LE week LE 7)) then stage_of_exercise=2; * missing;
if (id = 2689 and (5 LE week LE 6)) then stage_of_exercise=2; * missing;
if (id = 2689 and (9 LE week LE 10)) then stage_of_exercise=3; * missing;
if (id = 2691 and ( week = 8)) then stage_of_exercise=2; * missing;
if (id = 2691 and ( week = 9)) then stage_of_exercise=3; * missing;
array obs[4] obs1 - obs4; * minutes exercised at each exercise stage;
array exp[4] exp1 - exp4; * assigned days of exercise at each exercise stage;
array c[4] compl1-compl4; * percent minutes exercised/minutes assigned compliance at each exercise stage;
retain obs1 - obs4 exp1 - exp4 compl1-compl4 total_obs total_exp total_compl;
if (first.id=1) then do j = 1 to 4; obs[j] = 0; exp[j]=0; c[j]=0;
total_obs=0; total_exp=0; total_compl=0;
end;
total_obs = SUM(ExM, total_obs);
obs[stage_of_exercise] = SUM(ExM, obs[stage_of_exercise]);
if (last.week = 1) then do;
exp[stage_of_exercise] = SUM(exp[stage_of_exercise], Work_out_days_per_week);
total_exp = SUM(total_exp, Work_out_days_per_week);
end;
if (last.id=1) then do;
total_weeks_exercise = week;
final_stage_exercise = stage_of_exercise;
do j = 1 to 4;
  if (exp[j]>0) then  
c[j] = 100.0*obs[j]/(30.0*exp[j]);
  * percent minutes exercised/prescribed;
else c[j]= . ;
end;
if (total_exp > 0) then total_compl=100.0* total_obs/(30.0*total_exp);
else total_compl= . ;
output;
end;
keep id compl1-compl4 obs1 - obs4 exp1 - exp4 total_weeks_exercise final_stage_exercise total_obs total_exp total_compl;
proc sort data=wiser.exercise_compliance;
  by id;
proc print data=wiser.exercise_compliance(obs=20);
data wiser.adherence;
merge wiser.exercise_compliance wiser.srpa wiser.strata;
  by id;
exercise_complier = 0;
if (trt="exercise" and final_stage_exercise=4) then exercise_complier = 1;
D_srpa = total_mets_week_1 - total_mets_week_0;
if (trt="control" and D_srpa < 5.0) then exercise_complier = 1;
keep id exercise_complier;
run; quit;

/* Other problems in exercise logs:
2326 - missing
2367 - no logs
2508 - no logs
2510 - missing stage of exercise

/* proc contents data=wiser.exercise_logs;

AHR Num 8 average heart rate
CDM Num 8 cool down minutes
Date Num 8 workout date
Day Num 8 workout day on a given week
ExM Num 8 exercise minutes
ID Num 8 ID
Stage_of_exercise Num 8 Stage of exercise
THRhigh Num 8 higher target heart rate in the range
THRlow Num 8 lower target heart rate in the range
WUM Num 8 warm-up minutes
Week Num 8 week of exercise, ranges from 1 to 20
Work_out_days_per_week Num 8 expected workout days per week
* /

/*DATE:07/13/10
PURPOSE OF THIS CODE IS TO CALCULATE CHANGE IN MC BETWEEN CV7 AND CV2
CODE CATEGORIES:
A=4 MC between CV2 and CV7
B=3 MC between CV2 and CV7
C=5 MC between CV2 and CV7
D=6 MC between CV2 and CV7
E=2 MC between CV2 and CV7
F=Unknown

P3-P1 =1 E
P4-P1= 23 B
P5-P1= 261 A
P5-P1 =1 B
P5-P1=1 C
P6-P1= 19 C
P6-P1=4 A
P7-P1=3 D
P7-P1=1 C
*/

Data WISER.MC1;
INFILE 'D:\Users\Alma\Desktop\School\Menstrual logs\MClogs_data1.csv'
firstobs=2 DLM ="," DSD missover;

INPUT ID :$4.
CODE $1.
CODE_EXPLANATION :$56.
CYCLES $6.
;
FORMAT ID $4.
CODE $1.
CODE_EXPLANATION $56.
CYCLES $6.
;
RUN;

Data WISER.MC2;
INFILE 'D:\Users\Alma\Desktop\School\Menstrual logs\MClogs_data.csv'
firstobs=2 DLM ="," DSD missover;

INPUT ID :$4.
Start_P0 :MMDDYY10.
LH_P0 :MMDDYY10.
Start_P1 :MMDDYY10.
LH_P1 :MMDDYY10.
Start_P2 :MMDDYY10.
LH_P2    :MMDDYY10.
Start_P3 :MMDDYY10.
LH_P3    :MMDDYY10.
Start_P4 :MMDDYY10.
LH_P4    :MMDDYY10.
Start_P5 :MMDDYY10.
LH_P5    :MMDDYY10.
Start_P6 :MMDDYY10.
Start_P7 :MMDDYY10.
LH_P7    :MMDDYY10.
Start_P8 :MMDDYY10.
LH_P8    :MMDDYY10.

; 

FORMAT ID  S4.
Start_P0  MMDDYY10.
LH_P0     MMDDYY10.
    Start_P1 MMDDYY10.
LH_P1     MMDDYY10.
Start_P2  MMDDYY10.
LH_P2     MMDDYY10.
Start_P3  MMDDYY10.
LH_P3     MMDDYY10.
Start_P4  MMDDYY10.
LH_P4     MMDDYY10.
Start_P5  MMDDYY10.
LH_P5     MMDDYY10.
Start_P6  MMDDYY10.
LH_P6     MMDDYY10.
Start_P7  MMDDYY10.
LH_P7     MMDDYY10.
Start_P8  MMDDYY10.
LH_P8     MMDDYY10.

; run;

DATA WISER.MC2; /*Calculates MC length*/
set WISER.MC2;
MClength_P0= Start_P1- Start_P0;
MClength_P1= Start_P2- Start_P1;
MClength_P2= Start_P3- Start_P2;
MClength_P3= Start_P4- Start_P3;
MClength_P4= Start_P5- Start_P4;
MClength_P5= Start_P6- Start_P5;
MClength_P6= Start_P7- Start_P6;
MClength_P7= Start_P8- Start_P7;
run;

PROC SORT DATA=WISER.MC1;
BY ID;
PROC FREQ DATA=WISER.MC1;
table code;
run;
Proc Sort Data = WISER.MC2;
BY ID;
quit;

DATA WISER.MergedMC; /*Calculates change in MC length according to the above note*/
Merge WISER.MC1 WISER.MC2;
BY ID;
run;

DATA a;
set WISER.MergedMC;
where CODE = 'A';
MClength_0 = MClength_P1;
if Cycles = 'P5-P1' then MClength_1 = MClength_P5;
else if Cycles = 'P6-P1' then MClength_1 = MClength_P6;
D_MC = MClength_1 - MClength_0;
run;
proc print data=a;
var id MClength_P1 MClength_0 MClength_P5 MClength_P6 CYCLES MCLength_1 D_MC;
run;

DATA b;
set WISER.MergedMC;
where CODE = 'B';
MClength_0 = MClength_P1;
if Cycles = 'P4-P1' then MClength_1 = MClength_P4;
else if Cycles = 'P5-P1' then MClength_1 = MClength_P5;
D_MC = MClength_1 - MClength_0;
run;
proc print data=b;
var id MClength_P1 MClength_0 MClength_P4 MClength_P5 CYCLES MCLength_1 D_MC;
run;

DATA C;
set WISER.MergedMC;
where CODE = 'C';
MClength_0 = MClength_P1;
if Cycles = 'P6-P1' then MClength_1 = MClength_P6;
if Cycles = 'P7-P1' then MClength_1 = MClength_P7;
else if Cycles = 'P5-P1' then MClength_1 = MClength_P5;
D_MC = MClength_1 - MClength_0;
run;
proc print data=c;
var id MClength_P1 MClength_0 MClength_P5 MClength_P6 MClength_P7 CYCLES MCLength_1 D_MC;
run;

DATA DD;
set WISER.MergedMC;
where CODE = 'D';
MClength_0 = MClength_P1;
If Cycles = 'P7-P1' then MCLength_1 = MCLength_P7;
D_MC = MCLength_1 - MCLength_0;
RUN;

proc print data = DD;
var id MCLength_P1 MCLength_0 MCLength_P7 CYCLES MCLength_1 D_MC;
run;

DATA E;
set WISER.MergedMC;
where CODE = 'E';
MCLength_0 = MCLength_P1;
If Cycles = 'P3-P1' then MCLength_1 = MCLength_P3;
D_MC = MCLength_1 - MCLength_0;
RUN;

proc print data = E;
var id MCLength_P1 MCLength_0 MCLength_P3 CYCLES MCLength_1 D_MC;
run;

Data H;
set WISER.Demographics_319;
keep ID trt;
run;

Data WISER.ChangeMC;
merge A B C DD E H;
by id;
keep ID TRT MCLength_P1 MCLength_P2 MCLength_P3 MCLength_P4 MCLength_P5 MCLength_P6 MCLength_P7 CODE Cycles MCLength_0 MCLength_1 D_MC;
run;
proc print DATA = WISER.ChangeMC;
run;
pROC SORT DATA = WISER.CHANGEMC;
BY TRT;
RUN;

PROC MEANS DATA = WISER.CHANGEMC;
VAR MCLength_0 MCLength_1 D_MC;
BY trt;
RUN;

Proc GLM Data = wiser.changemc;
class trt;
model MCLength_0 MCLength_1 D_MC = trt ;
lsmeans trt / stderr pdiff;
run;

Data WISER.Merged_Data_319;
merge WISER.Demographics_319 WISER.Bodycomp_319 Wiser.Bodyweights_319 WISER.Menarche_310 WISER.Familyhistory_251 WISER.FoodRecords_312 WISER.Fitness_319 wiser.log_hormones_319 WISER.SRPA_319;
run;
Data WISER.Baseline_Associations;
Set WISER.Merged_Data_319;
KEEP trt age Ht.CV1 Weight_0 BMI_0 Percent_bodyfat_0 lean_mass_0 Menarche_age
   Kcal_0 Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 Caff_mg_0 Alc_g_0 Mets_0 total_mets_week_0
   log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 log_TT_0 log_BT_0 log_FT_0
   log_SHBG_0 log_P_0;
run;

/*Associations between hormones*/
Proc Corr Data=WISER.Baseline_Associations;
var log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 LOG_TT_0 LOG_BT_0 LOG_FT_0 LOG_SHBG_0
   LOG_P_0;
run;

/*Associations with baseline demographic factors*/
Proc Corr Data=WISER.Baseline_Associations;
var log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 LOG_TT_0 LOG_BT_0 LOG_FT_0 LOG_SHBG_0
   LOG_P_0;
with age Ht.CV1 weight_0 Percent_bodyfat_0 BMI_0 Menarche_age Kcal_0 Caff_mg_0 Alc_g_0
   Mets_0 total_mets_week_0;
run; quit;

/*Table 1. Baseline characteristics of Randomized participants (n=319)*/  

Data WISER.Table1;
Merge WISER.Demographics_319 WISER.Bodycomp_319 WISER.Menarche_310
   WISER.Familyhistory_251 WISER.FoodRecords_312 WISER.Fitness_319 wiser.srpa_319;
By id;
KEEP ID trt age age_strata Ht.CV1 Wt.CV1 fat_mass_0 Percent_bodyfat_0 lean_mass_0
   BMI BMI_strata BMI_class BMI_class4 BMI_over25 race Hispanic Education_cat MarStat status
   Menarche_age BC_history BCuse Parity
   Kcal_0 FatCals_0 Fib_g_0 Caff_mg_0 Alc_g_0 total_mets_week_0 Mets_0
; 
run;

Proc Ttest Data=wiser.Table1 ci=none;
class trt;
var age Ht.CV1 Wt.CV1 BMI fat_mass_0 Percent_bodyfat_0 lean_mass_0 Menarche_age Kcal_0
   Alc_g_0 Mets_0 total_mets_week_0;
run;
Proc Freq Data=wiser.Table1;
tables trt*(BMI_strata BMI_class4 race Hispanic Education_cat MarStat status BC_history
   BCuse Parity BMI_class age_strata)
/nocol chisq;
run;
/*Table 3. Changes in body weight, anthropometry, fitness, PA*/
Data WISER.Merged_Data_319;
Merge WISER.Demographics_319 WISER.Bodycomp_319 Wiser.Bodyweights_319
WISER.Menarche_310 WISER.Familyhistory_251
WISER.FoodRecords_312 WISER.Fitness_319 wiser.log_hormones_319 WISER.SRPA_319;
by id;
run;
/* trt* age_strata, trt*bmi_strata and trt*age_strata*bmi_strata interactions were not significant
so will be removed from model*/

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  model Mets_0 Mets_1 D_mets = trt bmi_strata | age_strata;
  lsmeans trt /stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  *model weight_0 weight_1 D_weight = trt | bmi_strata | age_strata;
  model weight_0 weight_1 D_weight= trt bmi_strata | age_strata;
  lsmeans trt /stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  *model bmi_0 bmi_1 D_bmi PD_bmi = trt | bmi_strata | age_strata;
  model bmi_0 bmi_1 D_bmi = trt bmi_strata | age_strata;
  lsmeans trt /stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  *model fat_mass_0 fat_mass_1 D_fat_mass PD_fat_mass= trt|bmi_strata|age_strata;
  model fat_mass_0 fat_mass_1 D_fat_mass = trt bmi_strata | age_strata;
  lsmeans trt /stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  *model percent_bodyfat_0 percent_bodyfat_1 D_percent_bodyfat PD_percent_bodyfat= 
  trt|bmi_strata|age_strata;
  model percent_bodyfat_0 percent_bodyfat_1 D_percent_bodyfat= trt bmi_strata | age_strata;
  lsmeans trt /stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
  class trt bmi_strata age_strata;
  *model percent_leanmass_0 percent_leanmass_1 D_percent_leanmass PD_percent_leanmass= 
  trt|bmi_strata|age_strata;
  model lean_mass_0 lean_mass_1 D_lean_mass = trt bmi_strata | age_strata;
Proc GLM Data=wiser.merged_data_319;
class trt bmi_strata age_strata;
model kcal_0 kcal_1 D_kcal = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
run;

Proc GLM Data=wiser.merged_data_319;
class trt bmi_strata age_strata;
model total_mets_week_0 total_mets_week_1 D_srpa = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
run;

Proc means data=wiser.merged_data_319 n min max mean;
var D_percent_bodyfat;
run;

proc univariate data=wiser.merged_data_319;
var D_percent_bodyfat;
run;

data x;
set wiser.merged_data_319;
if D_percent_bodyfat GE -2.0 and D_percent_bodyfat LE 2.0;
run;
proc print data=x;
var D_percent_bodyfat;
run;

******************************************************************************
All follicular completers n=319 (N=323 FOR BASELINE)
Purpose of this code: to check if there are differences at baseline between groups in hormone levels
Units for E2, BE2, FE2,FT = pg/ml
for TT, BT, and P=ng/ml
for SHBG = nmol/L
******************************************************************************
Data a;
set wiser.merged_data_319;
keep ID trt age_strata bmi_strata bmi_class;
run;

Data b;
set wiser.change_hormones_319;
keep ID D_E2 D_BE2 D_FE2 D_E1SO4 D_TT D_BT D_FT D_SHBG D_P;
RUN;

Data Wiser.Table3;
Merge a b wiser.log_hormones_319;
BY ID;
run;
/*Baseline comparisons strata adjusted: full model*
E1SO4_0 : trt p=0.0017 BMI_strata p =0.0107 trt*BMI_strata p=0.0156 and trt*age_strata*bmi_strata p=0.005
TT_0: age_strata p=0.002 bmi_strata p=0.001
BT_0: trt p=0.0412 age_strata AND bmi_strata p< 0.001
FT_0: age_strata p=0.003 bmi_strata p<0.001
SHBG_0: age_strata p=0.001 bmi_strata p<0.001 */

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 log_TT_0 log_BT_0 log_FT_0 log_SHBG_0 log_P_0=
  age_strata |bmi_strata | trt/ SS3;
run; quit;

/*Baseline comparisons strata adjusted: age and bmi interaction only log_E1SO4 includes trt*bmi interaction*/

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model log_E2_0 log_BE2_0 log_FE2_0 log_E1SO4_0 log_TT_0 log_BT_0 log_FT_0 log_SHBG_0 log_P_0=
  age_strata |bmi_strata | trt/ SS3;
lsmeans trt / stderr pdiff CL;
ODS output LSMeanCL=logCI_0;
run; quit;

Data b;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data=b;
run;

/*Follow up comparisons strata adjusted: age and bmi interactions
E1SO4_1 : trt p=0.0026 trt*BMI_strata p=0.035
TT_1*: age_strata p=0.0026, bmi_strata p =0.0007
BT_1,FT_1*: age_strata p=0.0002, bmi_strata p<00001, trt p=0.0255
SHBG_1*: age_strata p=0.0016, bmi_strata p<0.0001, age*bmi p=0.026
P_1 age_strata p=0.0183*/

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model log_E2_1 log_BE2_1 log_FE2_1 log_E1SO4_1 log_TT_1 log_BT_1 log_FT_1 log_SHBG_1 log_P_1=
  age_strata |bmi_strata | trt/ SS3;
run; quit;

/*Baseline comparisons strata adjusted: age and bmi interaction only log_E1SO4 includes trt*bmi interaction*/

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model log_E2_1 log_BE2_1 log_FE2_1 log_E1SO4_1 log_TT_1 log_BT_1 log_FT_1 log_SHBG_1 log_P_1= trt age_strata |bmi_strata/ SS3;
lsmeans trt / stderr pdiff CL;
ODS output LSMeanCL=logCI_2;
run;quit;

Data e;
Set logCI_2;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data= e;
run;

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model log_E1SO4_1= trt age_strata |bmi_strata trt*bmi_strata/ SS3;
lsmeans trt / stderr pdiff CL;
ODS output LSMeanCL=logCI_3;
run;quit;

Data f;
Set logCI_3;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data=f;
run;

/*Changes from baseline comparisons: age and bmi strata interaction*/

Proc GLM data=wiser.Table3;
class trt age_strata bmi_strata;
model D_E2 D_BE2 D_FE2 D_E1SO4 D_TT D_BT D_FT D_SHBG D_P = BMI_strata | age_strata trt/ SS3;
lsmeans trt/ stderr pdiff;
ods select LSMeans Diff;
run; quit;

Proc GLM data=Wiser.Table3; /*Reduced model*/
class trt age_strata bmi_strata;
model D_E1SO4 = BMI_strata | age_strata trt log_E1SO4_0/ SS3;
lsmeans trt/ stderr pdiff;
ods select LSMeans Diff;
run; quit;
APPENDIX C-2

SAS CODE: URINARY ESTROGEN METABOLITES
/*PURPOSE: to import creatinine csv datasheet and format it*/

Data EM.Creatinine;
INFILE 'D:\Users\Alma\Desktop\School\Estrogen Metabolites\Data\Creatinine.csv' firstobs=2 DLM =""," DSD missover LRECL= 1000 ;

INPUT ID      :$4.
                Pre_Cr_mgperdL   :3.
                Post_Cr_mgperdL :3.
                Pre_Cr_g        :4.2
                Post_Cr_g       :4.2
                Pre_Total_Vol   :5.
                Post_Total_Vol  :5.
                Pre_Total_Hrs   :5.2
                Post_Total_Hrs  :5.2;

FORMAT ID      $4.
                Pre_Cr_mgperdL   3.
                Post_Cr_mgperdL :3.
                Pre_Cr_g        4.2
                Post_Cr_g       4.2
                Pre_Total_Vol   5.
                Post_Total_Vol  5.
                Pre_Total_Hrs   5.2
                Post_Total_Hrs  5.2;

RUN;

/*PURPOSE: Import raw urine collection datasheet
   Calculate total urine collection time and volume
   Calculate total urine collection as ml of urine per day*/

Data EM.Tot_UrineVol;
INFILE 'D:\Users\Alma\Desktop\School\Estrogen Metabolites\Data\Tot_UrineVol.csv' firstobs=2 DLM =""," DSD missover LRECL= 1000 ;

INPUT ID          :$4.
                SHrs1pre       :Datetime18.
                EHrs1pre       :Datetime18.
                Vol1pre        :5.
                SHrs2pre       :Datetime18.
                EHrs2pre       :Datetime18.
                Vol2pre        :5.
                SHrs3pre       :Datetime18.
                EHrs3pre       :Datetime18.
                Vol3pre        :5.
                Comments_CV3   :$30.
                SHrs1fu        :Datetime18.
                EHrs1fu        :Datetime18.
                Vol1fu         :5.
/*Convert urine collection time from seconds to hours*/
Hrs1pre= (EHrs1pre - SHrs1pre)/3600; /*3600 = # seconds in an hr*/
Hrs2pre= (EHrs2pre - SHrs2pre)/3600;
Hrs3pre= (EHrs3pre - SHrs3pre)/3600;
Hrs1post= (EHrs1fu - SHrs1fu)/3600;
Hrs2post= (EHrs2fu - SHrs2fu)/3600;
Hrs3post= (EHrs3fu - SHrs3fu)/3600;

/*Calculate total urine collection time by adding time of day 1, 2, and 3*/
Pre_Hrs= SUM(Hrs1pre, Hrs2pre, Hrs3pre);
Post_Hrs= SUM(Hrs1post, Hrs2post, Hrs3post);

/*Based on notes from CV3 and CV8 raw spreadsheets the # of collection hours had to be changed*/
If ID='1093' then Pre_Hrs = Hrs2pre + Hrs3pre;
If ID='1517' then Pre_Hrs = Hrs2pre + Hrs3pre;
If ID='2438' then Pre_Hrs = 72.0;
If ID='2538' then Pre_Hrs = 72.0;

/*Convert total urine collection time from hours to days*/
Pre_Days = Pre_Hrs/24.0; /*24 = # hours in a day*/
Post_Days = Post_Hrs/24.0;

/*Calculate total urine collection volume by adding volumes of day 1, 2 and 3*/
Pre_Vol = SUM(Vol1pre, Vol2pre, Vol3pre);
Post_Vol = SUM(Vol1fu, Vol2fu, Vol3fu);

/*Calculate urine collection as ml per day*/
Pre_mLperday = Pre_Vol/Pre_Days;
Post_mLperday = Post_Vol/Post_Days;

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<td>Vol2pre</td>
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<tr>
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</table>
Pre_Days 3.1
Post_Days 3.1
Pre_Vol 5.1
Post_Vol 5.1
Pre_mLperday 6.1
Post_mLperday 6.1

; run;

proc print;
run;

/*PURPOSE: Import raw EM datasheet */
/*NOTE: Urine concentrations in EM_318.csv are in ng/ml urine and ND samples are read as missing
ID 2622 finished CV7 but not CV8 and therefore excluded*/

Data EM.EM_ngpermL_original;
INFILE 'D:\Users\Alma\Desktop\School\Estrogen Metabolites\Data\EM_318.csv'
firstobs=2 DLM = "", DSD missover;
INPUT ID :$4.
         E1_0  : 8.3
         E1_1  : 8.3
         E2_0  : 8.3
         E2_1  : 8.3
         E3_0  : 8.3
         E3_1  : 8.3
         _16_OH_E1_0 : 8.3
         _16_OH_E1_1 : 8.3
         OH_2_E1_0   : 8.3
         OH_2_E1_1   : 8.3
         OH_4_E1_0   : 8.3
         OH_4_E1_1   : 8.3
         OH_2_E2_0   : 8.3
         OH_2_E2_1   : 8.3
         OH_4_E2_0   : 8.3
         OH_4_E2_1   : 8.3
         MeO_2_E1_0  : 8.3
         MeO_2_E1_1  : 8.3
         MeO_4_E1_0  : 8.3
         MeO_4_E1_1  : 8.3
         MeO_2_E2_0  : 8.3
         MeO_2_E2_1  : 8.3
         MeO_4_E2_0  : 8.3
         MeO_4_E2_1  : 8.3
;

FORMAT ID $4.
         E1_0  8.3
         E1_1  8.3
         E2_0  8.3
         E2_1  8.3
proc means n nmiss min max;
/*There are missing values for all EM*/
run;

/*PURPOSE: Per MK, WT, and WP, missing datapoints are to be assigned the lowest standard value (0.014ng/ml)*/
Data EM.EM_ngperml_LowSTD;
Set EM.EM_ngperml_original;

If E1_0 = . then E1_0=0.014;
If E1_1 = . then E1_1=0.014;
If E2_0 = . then E2_0=0.014;
If E2_1 = . then E2_1=0.014;
If E3_0 = . then E3_0=0.014;
If E3_1 = . then E3_1=0.014;
If _16_OH_E1_0 = . then _16_OH_E1_0=0.014;
If _16_OH_E1_1 = . then _16_OH_E1_1=0.014;
If OH_2_E1_0 = . then OH_2_E1_0=0.014;
If OH_2_E1_1 = . then OH_2_E1_1=0.014;
If OH_4_E1_0 = . then OH_4_E1_0=0.014;
If OH_4_E1_1 = . then OH_4_E1_1=0.014;
If OH_2_E2_0 = . then OH_2_E2_0=0.014;
If OH_2_E2_1 = . then OH_2_E2_1=0.014;
If OH_4_E2_0 = . then OH_4_E2_0=0.014;
If OH_4_E2_1 = . then OH_4_E2_1=0.014;
If MeO_2_E1_0 = . then MeO_2_E1_0 =0.014;
If MeO_2_E1_1 = . then MeO_2_E1_1 =0.014;
If MeO_4_E1_0 = . then MeO_4_E1_0 =0.014;
If MeO_4_E1_1 = . then MeO_4_E1_1 =0.014;
If MeO_2_E2_0 = . then MeO_2_E2_0 =0.014;
If MeO_2_E2_1 = . then MeO_2_E2_1 =0.014;
If MeO_4_E2_0 = . then MeO_4_E2_0 = 0.014;
If MeO_4_E2_1 = . then MeO_4_E2_1 = 0.014;
run;

proc means;
run; /*All previously missing values have now been re-assigned 0.014ng/ml values*/

proc univariate plot Data=EM.EM_ngperml_LowSTD;
/*all EM are right-skewed so will need to take logs*/
run;
proc print;
run;

/*PURPOSE: Convert ng EM/ml urine into ng EM/mg Cr */

Data EM.EM_ngpermgCr_LowSTD;
Merge EM.EM_ngperml_LowSTD EM.Creatinine EM.Tot_UrineVol;
Drop Pre_Cr_g Post_Cr_g Pre_Total_Vol Post_Total_Vol Pre_Total_Hrs Post_Total_Hrs SHrs1pre EHrs1pre Vol1pre SHrs2pre EHrs2pre Vol2pre SHrs3pre EHrs3pre Vol3pre Comments_CV3 SHrs1fu EHrs1fu Vol1fu SHrs2fu EHrs2fu Vol2fu SHrs3fu EHrs3fu Vol3fu Hrs1pre Hrs2pre Hrs3pre Pre_hrs Pre_days Hrs1post Hrs2post Hrs3post Post_hrs Post_days;

/*((ng EM/mL urine)*(100mL/1dL))/(mg Cr/dL urine)*/
E1_0_ngpermgCr = (E1_0*100)/Pre_Cr_mgperdL;
E1_1_ngpermgCr = (E1_1*100)/Post_Cr_mgperdL;
E2_0_ngpermgCr = (E2_0*100)/Pre_Cr_mgperdL;
E2_1_ngpermgCr = (E2_1*100)/Post_Cr_mgperdL;
E3_0_ngpermgCr = (E3_0*100)/Pre_Cr_mgperdL;
E3_1_ngpermgCr = (E3_1*100)/Post_Cr_mgperdL;
_16_OH_E1_0_ngpermgCr= (_16_OH_E1_0*100)/Pre_Cr_mgperdL;
_16_OH_E1_1_ngpermgCr= (_16_OH_E1_1*100)/Post_Cr_mgperdL;
OH_2_E1_0_ngpermgCr = (OH_2_E1_0*100)/Pre_Cr_mgperdL;
OH_2_E1_1_ngpermgCr = (OH_2_E1_1*100)/Post_Cr_mgperdL;
OH_4_E1_0_ngpermgCr = (OH_4_E1_0*100)/Pre_Cr_mgperdL;
OH_4_E1_1_ngpermgCr = (OH_4_E1_1*100)/Post_Cr_mgperdL;
OH_2_E2_0_ngpermgCr = (OH_2_E2_0*100)/Pre_Cr_mgperdL;
OH_2_E2_1_ngpermgCr = (OH_2_E2_1*100)/Post_Cr_mgperdL;
OH_4_E2_0_ngpermgCr = (OH_4_E2_0*100)/Pre_Cr_mgperdL;
OH_4_E2_1_ngpermgCr = (OH_4_E2_1*100)/Post_Cr_mgperdL;
MeO_2_E1_0_ngpermgCr= (MeO_2_E1_0*100)/Pre_Cr_mgperdL;
MeO_2_E1_1_ngpermgCr= (MeO_2_E1_1*100)/Post_Cr_mgperdL;
MeO_4_E1_0_ngpermgCr= (MeO_4_E1_0*100)/Pre_Cr_mgperdL;
MeO_4_E1_1_ngpermgCr= (MeO_4_E1_1*100)/Post_Cr_mgperdL;
MeO_2_E2_0_ngpermgCr= (MeO_2_E2_0*100)/Pre_Cr_mgperdL;
MeO_2_E2_1_ngpermgCr= (MeO_2_E2_1*100)/Post_Cr_mgperdL;
MeO_4_E2_0_ngpermgCr= (MeO_4_E2_0*100)/Pre_Cr_mgperdL;
MeO_4_E2_1_ngpermgCr= (MeO_4_E2_1*100)/Post_Cr_mgperdL;

FORMAT ID $4.
   E1_0_ngpermgCr 6.3
   E1_1_ngpermgCr 6.3
   E2_0_ngpermgCr 6.3
proc means n min;
var E1_0 ngpermgCr E1_1 ngpermgCr E2_0 ngpermgCr E2_1 ngpermgCr E3_0 ngpermgCr E3_1 ngpermgCr _16_OH_E1_0 ngpermgCr _16_OH_E1_1 ngpermgCr OH_2_E1_0 ngpermgCr OH_2_E1_1 ngpermgCr OH_4_E1_0 ngpermgCr OH_4_E1_1 ngpermgCr OH_4_E2_0 ngpermgCr OH_4_E2_1 ngpermgCr OH_2_E2_0 ngpermgCr OH_2_E2_1 ngpermgCr OH_4_E2_0 ngpermgCr OH_4_E2_1 ngpermgCr MeO_2_E1_0 ngpermgCr MeO_2_E1_1 ngpermgCr MeO_4_E1_0 ngpermgCr MeO_4_E1_1 ngpermgCr MeO_4_E2_0 ngpermgCr MeO_4_E2_1 ngpermgCr;
run;
/*PURPOSE: To convert ng EM/ml urine into nmol/day*/
Data EM.EM_nmolperday_LowSTD;
Merge EM.EM_ngpermL_LowSTD EM.Tot_UrineVol;
Keep ID Pre_mLperday Post_mLperday E1_0 E1_1 E2_0 E2_1 E3_0 E3_1 _16_OH_E1_0 _16_OH_E1_1 OH_2_E1_0 OH_2_E1_1 OH_2_E2_0 OH_2_E2_1 OH_4_E1_0 OH_4_E1_1 OH_4_E2_0 OH_4_E2_1 MeO_2_E1_0 MeO_2_E1_1 MeO_2_E2_0 MeO_2_E2_1 MeO_4_E1_0 MeO_4_E1_1 MeO_4_E2_0 MeO_4_E2_1;
run;
/*PURPOSE: Convert concentration from mg/day to nmol/day*/
Data EM.EM_nmolperday_LowSTD;
Set EM.EM_nmolperday_LowSTD;
E1_0_nmolperday = (E1_0/270.37)*Pre_mLperday;
E1_1_nmolperday = (E1_1/270.37)*Post_mLperday;
E2_0_nmolperday = (E2_0/272.38)*Pre_mLperday;
E2_1_nmolperday = (E2_1/272.38)*Post_mLperday;
E3_0_nmolperday = (E3_0/288.38)*Pre_mLperday;
E3_1_nmolperday = (E3_1/288.38)*Post_mLperday;
_16_OH_E1_0_nmolperday = (_16_OH_E1_0/286.37)*Pre_mLperday;
_16_OH_E1_1_nmolperday = (_16_OH_E1_1/286.37)*Post_mLperday;
OH_2_E1_0_nmolperday = (OH_2_E1_0/286.37)*Pre_mLperday;
OH_2_E1_1_nmolperday = (OH_2_E1_1/286.37)*Post_mLperday;
OH_4_E1_0_nmolperday = (OH_4_E1_0/286.37)*Pre_mLperday;
OH_4_E1_1_nmolperday = (OH_4_E1_1/286.37)*Post_mLperday;
OH_2_E2_0_nmolperday = (OH_2_E2_0/288.37)*Pre_mLperday;
OH_2_E2_1_nmolperday = (OH_2_E2_1/288.37)*Post_mLperday;
OH_4_E2_0_nmolperday = (OH_4_E2_0/288.37)*Pre_mLperday;
OH_4_E2_1_nmolperday = (OH_4_E2_1/288.37)*Post_mLperday;
MeO_2_E1_0_nmolperday = (MeO_2_E1_0/300.39)*Post_mLperday;
MeO_2_E1_1_nmolperday = (MeO_2_E1_1/300.39)*Pre_mLperday;
MeO_4_E1_0_nmolperday = (MeO_4_E1_0/300.39)*Pre_mLperday;
MeO_4_E1_1_nmolperday = (MeO_4_E1_1/300.39)*Post_mLperday;
MeO_2_E2_0_nmolperday = (MeO_2_E2_0/302.41)*Pre_mLperday;
MeO_2_E2_1_nmolperday = (MeO_2_E2_1/302.41)*Post_mLperday;
MeO_4_E2_0_nmolperday = (MeO_4_E2_0/302.41)*Pre_mLperday;
MeO_4_E2_1_nmolperday = (MeO_4_E2_1/302.41)*Post_mLperday;

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DROP Pre_mLperday Post_mLperday
/*PURPOSE: Calculate EM ratios (ng EM/ml urine)*/
Data EM.Ratios_EM_ngperml_LowSTD;
Set EM.EM_ngperml_LowSTD;

R_2to16E1_0 = OH_2_E1_0/16_OH_E1_0;
R_2to16E1_1 = OH_2_E1_1/16_OH_E1_1;
R_2to4E1_0 = OH_2_E1_0/OH_4_E1_0;
R_2to4E1_1 = OH_2_E1_1/OH_4_E1_1;
Total_2OHE_0 = (OH_2_E1_0 + OH_2_E2_0);
Total_2OHE_1 = (OH_2_E1_1 + OH_2_E2_1);
Total_4OHE_0 = (OH_4_E1_0 + OH_4_E2_0);
Total_4OHE_1 = (OH_4_E1_1 + OH_4_E2_1);
Total_2MeOE_0 = (MeO_2_E1_0 + MeO_2_E2_0);
Total_2MeOE_1 = (MeO_2_E1_1 + MeO_2_E2_1);
Total_4MeOE_0 = (MeO_4_E1_0 + MeO_4_E2_0);
Total_4MeOE_1 = (MeO_4_E1_1 + MeO_4_E2_1);
R_total_2to4OHE_0 = (Total_2OHE_0/Total_4OHE_0);
R_total_2to4OHE_1 = (Total_2OHE_1/Total_4OHE_1);
R_total_2to4MeOE_0 = (Total_2MeOE_0/Total_4MeOE_0);
R_total_2to4MeOE_1 = (Total_2MeOE_1/Total_4MeOE_1);

FORMAT
R_2to16E1_0 8.3
R_2to16E1_1 8.3
R_2to4E1_0 8.3
R_2to4E1_1 8.3
Total_2OHE_0 8.3
Total_2OHE_1 8.3
Total_4OHE_0 8.3
Total_4OHE_1 8.3
Total_2MeOE_0 8.3
Total_2MeOE_1 8.3
Total_4MeOE_0 8.3
Total_4MeOE_1 8.3
R_total_2to4OHE_0 8.3
R_total_2to4OHE_1 8.3
R_total_2to4MeOE_0 8.3
R_total_2to4MeOE_1 8.3
;
RUN;

proc means data=em.Ratios_EM_ngperml_LowSTD;
/*RESULT: All hormones are right-skewed (max/min >10) so need to log transform*/

Data EM.Log_EM_ngperml_LowSTD;
Set EM.Ratios_EM_ngperml_LowSTD;

log_E1_0 = log(E1_0);
log_E1_1 = log(E1_1);
log_E2_0 = log(E2_0);
log_E2_1 = log(E2_1);
log_E3_0 = log(E3_0);
log_E3_1 = log(E3_1);
log_16_OH_E1_0 = log(16_OH_E1_0);
log_16_OH_E1_1 = log(16_OH_E1_1);
log_OH_2_E1_0 = log(OH_2_E1_0);
log_OH_2_E1_1 = log(OH_2_E1_1);
log_OH_4_E1_0 = log(OH_4_E1_0);
log_OH_4_E1_1 = log(OH_4_E1_1);
log_OH_2_E2_0 = log(OH_2_E2_0);
log_OH_2_E2_1 = log(OH_2_E2_1);
log_OH_4_E2_0 = log(OH_4_E2_0);
log_OH_4_E2_1 = log(OH_4_E2_1);
log_MeO_2_E1_0 = log(MeO_2_E1_0);
log_MeO_2_E1_1 = log(MeO_2_E1_1);
log_MeO_4_E1_0 = log(MeO_4_E1_0);
log_MeO_4_E1_1 = log(MeO_4_E1_1);
log_MeO_2_E2_0 = log(MeO_2_E2_0);
log_MeO_2_E2_1 = log(MeO_2_E2_1);
log_MeO_4_E2_0 = log(MeO_4_E2_0);
log_MeO_4_E2_1 = log(MeO_4_E2_1);
log_R_2to16E1_0 = log(R_2to16E1_0);
log_R_2to16E1_1 = log(R_2to16E1_1);
log_R_2to4E1_0 = log(R_2to4E1_0);
log_R_2to4E1_1 = log(R_2to4E1_1);
log_R_total_2to4OHE_0 = log(R_total_2to4OHE_0);
log_R_total_2to4OHE_1 = log(R_total_2to4OHE_1);
log_R_total_2to4MeOE_0 = log(R_total_2to4MeOE_0);
log_R_total_2to4MeOE_1 = log(R_total_2to4MeOE_1);
log_Total_2OHE_0 = log(Total_2OHE_0);
log_Total_2OHE_1 = log(Total_2OHE_1);
log_Total_4OHE_0 = log(Total_4OHE_0);
log_Total_4OHE_1 = log(Total_4OHE_1);
log_Total_2MeOE_0 = log(Total_2MeOE_0);
log_Total_2MeOE_1 = log(Total_2MeOE_1);
log_Total_4MeOE_0 = log(Total_4MeOE_0);
log_Total_4MeOE_1 = log(Total_4MeOE_1);

Format
log_E1_0 8.3
log_E1_1 8.3
log_E2_0 8.3
log_E2_1 8.3
log_E3_0 8.3
log_E3_1  8.3
log_16_OH_E1_0  8.3
log_16_OH_E1_1  8.3
log_OH_2_E1_0  8.3
log_OH_2_E1_1  8.3
log_OH_4_E1_0  8.3
log_OH_4_E1_1  8.3
log_OH_2_E2_0  8.3
log_OH_2_E2_1  8.3
log_OH_4_E2_0  8.3
log_OH_4_E2_1  8.3
log_MeO_2_E1_0  8.3
log_MeO_2_E1_1  8.3
log_MeO_4_E1_0  8.3
log_MeO_4_E1_1  8.3
log_MeO_2_E2_0  8.3
log_MeO_2_E2_1  8.3
log_MeO_4_E2_0  8.3
log_MeO_4_E2_1  8.3
log_R_2to16E1_0  8.3
log_R_2to16E1_1  8.3
log_R_2to4E1_0  8.3
log_R_2to4E1_1  8.3
log_R_total_2to4OHE_0  8.3
log_R_total_2to4OHE_1  8.3
log_R_total_2to4MeOE_0  8.3
log_R_total_2to4MeOE_1  8.3
log_Total_2OHE_0  8.3
log_Total_2OHE_1  8.3
log_Total_4OHE_0  8.3
log_Total_4OHE_1  8.3
log_Total_2MeOE_0  8.3
log_Total_2MeOE_1  8.3
log_Total_4MeOE_0  8.3
log_Total_4MeOE_1  8.3;
run;

/*PURPOSE: Calculate change in concentration*/
Data EM.D_EM_ngperml_LowSTD;
Set EM.Log_EM_ngperml_LowSTD;

array a[20] E1_0 E2_0 E3_0 _16_OH_E1_0
          OH_2_E1_0 OH_4_E1_0 OH_2_E2_0 OH_4_E2_0
          MeO_2_E1_0 MeO_4_E1_0 MeO_2_E2_0 MeO_4_E2_0
          R_2to16E1_0 R_2to4E1_0 R_total_2to4OHE_0 R_total_2to4MeOE_0
          Total_2OHE_0 Total_4OHE_0
          Total_2MeOE_0 Total_4MeOE_0;
array b[20] E1_1 E2_1 E3_1 _16_OH_E1_1
          OH_2_E1_1 OH_4_E1_1 OH_2_E2_1 OH_4_E2_1
          MeO_2_E1_1 MeO_4_E1_1 MeO_2_E2_1 MeO_4_E2_1

run;
R_2to16E1_1 R_2to4E1_1
R_total_2to4OHE_1 R_total_2to4MeOE_1
Total_2OHE_1 Total_4OHE_1
Total_2MeOE_1 Total_4MeOE_1;

array c[20] D_E1 D_E2 D_E3 D__16_OH_E1
    D_OH_2_E1 D_OH_4_E1 D_OH_2_E2 D_OH_4_E2
    D_MeO_2_E1 D_MeO_4_E1 D_MeO_2_E2 D_MeO_4_E2
    D_R_2to16E1 D_R_2to4E1
    D_R_total_2to4OHE D_R_total_2to4MeOE
    D_Total_2OHE D_Total_4OHE
    D_Total_2MeOE D_Total_4MeOE;

do j=1 to 20;
c[j]= b[j]-a[j];
end;

Format D_E1 8.3
    D_E2 8.3
    D_E3 8.3
    D_MeO_2_E1 8.3
    D_MeO_4_E1 8.3
    D_MeO_2_E2 8.3
    D_MeO_4_E2 8.3
    D__16_OH_E1 8.3
    D_OH_2_E1 8.3
    D_OH_4_E1 8.3
    D_OH_2_E2 8.3
    D_OH_4_E2 8.3
    D_R_2to16E1 8.3
    D_R_2to4E1 8.3
    D_Total_2OHE 8.3
    D_Total_4OHE 8.3
    D_Total_2MeOE 8.3
    D_Total_4MeOE 8.3
    D_R_total_2to4OHE 8.3
    D_R_total_2to4MeOE 8.3;

RUN;

proc means DATA=EM.D_EM_ngperml_LowSTD; RUN;

/*PURPOSE: Check the distribution of data, if skewed then need to log transform*/
proc univariate plot data=EM.D_EM_ngperml_LowSTD;
var D_E1 D_E2 D_E3 D__16_OH_E1
    D_OH_2_E1 D_OH_4_E1 D_OH_2_E2 D_OH_4_E2
    D_MeO_2_E1 D_MeO_4_E1 D_MeO_2_E2 D_MeO_4_E2
    D_R_2to16E1 D_R_2to4E1 D_R_total_2to4OHE D_R_total_2to4MeOE
    D_Total_2OHE D_Total_2MeOE D_Total_4OHE D_Total_4MeOE;
/*Distributions are normally distributed so no need to log transform changes from baseline*/
run;
Purpose: Merge all datasets into one

```r
/* PURPOSE: Merge all datasets into one*/
Data Merged_A_LowSTD;
Merge EM.Ratios_EM_ngperml_LowSTD EM.D_EM_ngperml_LowSTD
EM.LOG_EM_ngperml_LowSTD;
RUN;
```

Purpose: Create a dataset with ID, trt, age_strata and bmi_strata to merge to EM.EM_318

```r
/*Create a dataset with ID, trt, age_strata and bmi_strata to merge to EM.EM_318*/
Data Merged_B_LowSTD;
Set WISER.Table1;
keep id trt age_strata bmi_strata bmi_class bmi_over25;
run;
```

Purpose: Add to merged data set id, trt and age & bmi strata

```r
/*PURPOSE: Add to merged data set id, trt and age & bmi strata*/
Data EM.EM_Merged_ngperml_LowSTD;
Merge Merged_B_LowSTD Merged_A_LowSTD;
RUN;
```

Purpose: Calculate EM ratios (ng EM/mg Cr)

```r
/*PURPOSE: Calculate EM ratios (ng EM/mg Cr)*/
Data EM.Ratios_EM_ngpermgCr_LowSTD;
Set EM.EM_ngpermgCr_LowSTD;

R_2to16E1_0_ngpermgCr = OH_2_E1_0_ngpermgCr/16_OH_E1_0_ngpermgCr;
R_2to16E1_1_ngpermgCr = OH_2_E1_1_ngpermgCr/16_OH_E1_1_ngpermgCr;
R_2to4E1_0_ngpermgCr = OH_2_E1_0_ngpermgCr/OH_4_E1_0_ngpermgCr;
R_2to4E1_1_ngpermgCr = OH_2_E1_1_ngpermgCr/OH_4_E1_1_ngpermgCr;
Total_2OHE_0_ngpermgCr = (OH_2_E1_0_ngpermgCr + OH_2_E2_0_ngpermgCr);
Total_2OHE_1_ngpermgCr = (OH_2_E1_1_ngpermgCr + OH_2_E2_1_ngpermgCr);
Total_4OHE_0_ngpermgCr = (OH_4_E1_0_ngpermgCr + OH_4_E2_0_ngpermgCr);
Total_4OHE_1_ngpermgCr = (OH_4_E1_1_ngpermgCr + OH_4_E2_1_ngpermgCr);
Total_2MeOE_0_ngpermgCr = (MeO_2_E1_0_ngpermgCr + MeO_2_E2_0_ngpermgCr);
Total_2MeOE_1_ngpermgCr = (MeO_2_E1_1_ngpermgCr + MeO_2_E2_1_ngpermgCr);
Total_4MeOE_0_ngpermgCr = (MeO_4_E1_0_ngpermgCr + MeO_4_E2_0_ngpermgCr);
Total_4MeOE_1_ngpermgCr = (MeO_4_E1_1_ngpermgCr + MeO_4_E2_1_ngpermgCr);
```
Total_4MeOE_1_ngpermgCr = (MeO_4_E1_1_ngpermgCr + MeO_4_E2_1_ngpermgCr);
R_tot_2to4OHES_0_ngpermgCr = (Total_2OHES_0_ngpermgCr/Total_4OHES_0_ngpermgCr);
R_tot_2to4OHES_1_ngpermgCr = (Total_2OHES_1_ngpermgCr/Total_4OHES_1_ngpermgCr);
R_tot_2to4MeOES_0_ngpermgCr = (Total_2MeOES_0_ngpermgCr/Total_4MeOES_0_ngpermgCr);
R_tot_2to4MeOES_1_ngpermgCr = (Total_2MeOES_1_ngpermgCr/Total_4MeOES_1_ngpermgCr);

FORMAT
R_2to16E1_0_ngpermgCr 8.3
R_2to16E1_1_ngpermgCr 8.3
R_2to4E1_0_ngpermgCr 8.3
R_2to4E1_1_ngpermgCr 8.3
Total_2OHES_0_ngpermgCr 8.3
Total_2OHES_1_ngpermgCr 8.3
Total_4OHES_0_ngpermgCr 8.3
Total_4OHES_1_ngpermgCr 8.3
Total_2MeOES_0_ngpermgCr 8.3
Total_2MeOES_1_ngpermgCr 8.3
Total_4MeOES_0_ngpermgCr 8.3
Total_4MeOES_1_ngpermgCr 8.3
R_tot_2to4OHES_0_ngpermgCr 8.3
R_tot_2to4OHES_1_ngpermgCr 8.3
R_tot_2to4MeOES_0_ngpermgCr 8.3
R_tot_2to4MeOES_1_ngpermgCr 8.3;

DROP E1_0 E1_1 E2_0 E2_1
E3_0 E3_1 _16_OH_E1_0 _16_OH_E1_1
OH_2_E1_0 OH_2_E1_1 OH_2_E2_0 OH_2_E2_1
OH_4_E1_0 OH_4_E1_1 OH_4_E2_0 OH_4_E2_1
MeO_2_E1_0 MeO_2_E1_1 MeO_2_E2_0 MeO_2_E2_1
MeO_4_E1_0 MeO_4_E1_1 MeO_4_E2_0 MeO_4_E2_1;
RUN;
proc means data=em.Ratios_EM_ngpermgCr_LowSTD;
run;
/*RESULT: All hormones are right-skewed (max/min >10) so need to log transform*/
Data EM.Log_EM_ngpermgCr_LowSTD;
Set EM.Ratios_EM_ngpermgCr_LowSTD;
log_E1_0_ngpermgCr = log(E1_0_ngpermgCr);
log_E1_1_ngpermgCr = log(E1_1_ngpermgCr);
log_E2_0_ngpermgCr = log(E2_0_ngpermgCr);
log_E2_1_ngpermgCr = log(E2_1_ngpermgCr);
log_E3_0_ngpermgCr = log(E3_0_ngpermgCr);
log_E3_1_ngpermgCr = log(E3_1_ngpermgCr);
log__16_OH_E1_0_ngpermgCr = log(_16_OH_E1_0_ngpermgCr);
log__16_OH_E1_1_ngpermgCr = log(_16_OH_E1_1_ngpermgCr);
log_OH_2_E1_0_ngpermgCr = log(OH_2_E1_0_ngpermgCr);
log_OH_2_E1_1_ngpermgCr = log(OH_2_E1_1_ngpermgCr);
log_OH_4_E1_0_ngpermgCr = log(OH_4_E1_0_ngpermgCr);
log_OH_4_E1_1_ngpermgCr = log(OH_4_E1_1_ngpermgCr);
log_OH_2_E2_0_ngpermgCr = log(OH_2_E2_0_ngpermgCr);
log_OH_2_E2_1_ngpermgCr = log(OH_2_E2_1_ngpermgCr);
log_OH_4_E2_0_ngpermgCr = log(OH_4_E2_0_ngpermgCr);
log_OH_4_E2_1_ngpermgCr = log(OH_4_E2_1_ngpermgCr);
log_MeO_2_E1_0_ngpermgCr = log(MeO_2_E1_0_ngpermgCr);
log_MeO_2_E1_1_ngpermgCr = log(MeO_2_E1_1_ngpermgCr);
log_MeO_4_E1_0_ngpermgCr = log(MeO_4_E1_0_ngpermgCr);
log_MeO_4_E1_1_ngpermgCr = log(MeO_4_E1_1_ngpermgCr);
log_MeO_2_E2_0_ngpermgCr = log(MeO_2_E2_0_ngpermgCr);
log_MeO_2_E2_1_ngpermgCr = log(MeO_2_E2_1_ngpermgCr);
log_MeO_4_E2_0_ngpermgCr = log(MeO_4_E2_0_ngpermgCr);
log_MeO_4_E2_1_ngpermgCr = log(MeO_4_E2_1_ngpermgCr);
log_R_2to16E1_0_ngpermgCr = log(R_2to16E1_0_ngpermgCr);
log_R_2to16E1_1_ngpermgCr = log(R_2to16E1_1_ngpermgCr);
log_R_2to4E1_0_ngpermgCr = log(R_2to4E1_0_ngpermgCr);
log_R_2to4E1_1_ngpermgCr = log(R_2to4E1_1_ngpermgCr);
log_R_tot_2to4OHE_0_ngpermgCr = log(R_tot_2to4OHE_0_ngpermgCr);
log_R_tot_2to4OHE_1_ngpermgCr = log(R_tot_2to4OHE_1_ngpermgCr);
log_R_tot_2to4MeOE_0_ngpermgCr = log(R_tot_2to4MeOE_0_ngpermgCr);
log_R_tot_2to4MeOE_1_ngpermgCr = log(R_tot_2to4MeOE_1_ngpermgCr);
log_Total_2OHE_0_ngpermgCr = log(Total_2OHE_0_ngpermgCr);
log_Total_2OHE_1_ngpermgCr = log(Total_2OHE_1_ngpermgCr);
log_Total_4OHE_0_ngpermgCr = log(Total_4OHE_0_ngpermgCr);
log_Total_4OHE_1_ngpermgCr = log(Total_4OHE_1_ngpermgCr);
log_Total_2MeOE_0_ngpermgCr = log(Total_2MeOE_0_ngpermgCr);
log_Total_2MeOE_1_ngpermgCr = log(Total_2MeOE_1_ngpermgCr);
log_Total_4MeOE_0_ngpermgCr = log(Total_4MeOE_0_ngpermgCr);
log_Total_4MeOE_1_ngpermgCr = log(Total_4MeOE_1_ngpermgCr);

Format
log_E1_0_ngpermgCr  8.3
log_E1_1_ngpermgCr  8.3
log_E2_0_ngpermgCr  8.3
log_E2_1_ngpermgCr  8.3
log_E3_0_ngpermgCr  8.3
log_E3_1_ngpermgCr  8.3
log_OH_2_E1_0_ngpermgCr  8.3
log_OH_2_E1_1_ngpermgCr  8.3
log_OH_4_E1_0_ngpermgCr  8.3
log_OH_4_E1_1_ngpermgCr  8.3
log_OH_2_E2_0_ngpermgCr  8.3
log_OH_2_E2_1_ngpermgCr  8.3
log_OH_4_E2_0_ngpermgCr  8.3
log_OH_4_E2_1_ngpermgCr  8.3
log_MeO_2_E1_0_ngpermgCr  8.3
log_MeO_2_E1_1_ngpermgCr  8.3
log_MeO_4_E1_0_ngpermgCr  8.3
log_MeO_4_E1_1_ngpermgCr  8.3
log_MeO_2_E2_0_ngpermgCr  8.3
log_MeO_2_E2_1_ngpermgCr  8.3
log_MeO_4_E2_0_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3

Format
log_E1_0_ngpermgCr  8.3
log_E1_1_ngpermgCr  8.3
log_E2_0_ngpermgCr  8.3
log_E2_1_ngpermgCr  8.3
log_E3_0_ngpermgCr  8.3
log_E3_1_ngpermgCr  8.3
log_OH_2_E1_0_ngpermgCr  8.3
log_OH_2_E1_1_ngpermgCr  8.3
log_OH_4_E1_0_ngpermgCr  8.3
log_OH_4_E1_1_ngpermgCr  8.3
log_OH_2_E2_0_ngpermgCr  8.3
log_OH_2_E2_1_ngpermgCr  8.3
log_OH_4_E2_0_ngpermgCr  8.3
log_OH_4_E2_1_ngpermgCr  8.3
log_MeO_2_E1_0_ngpermgCr  8.3
log_MeO_2_E1_1_ngpermgCr  8.3
log_MeO_4_E1_0_ngpermgCr  8.3
log_MeO_4_E1_1_ngpermgCr  8.3
log_MeO_2_E2_0_ngpermgCr  8.3
log_MeO_2_E2_1_ngpermgCr  8.3
log_MeO_4_E2_0_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3

Format
log_E1_0_ngpermgCr  8.3
log_E1_1_ngpermgCr  8.3
log_E2_0_ngpermgCr  8.3
log_E2_1_ngpermgCr  8.3
log_E3_0_ngpermgCr  8.3
log_E3_1_ngpermgCr  8.3
log_OH_2_E1_0_ngpermgCr  8.3
log_OH_2_E1_1_ngpermgCr  8.3
log_OH_4_E1_0_ngpermgCr  8.3
log_OH_4_E1_1_ngpermgCr  8.3
log_OH_2_E2_0_ngpermgCr  8.3
log_OH_2_E2_1_ngpermgCr  8.3
log_OH_4_E2_0_ngpermgCr  8.3
log_OH_4_E2_1_ngpermgCr  8.3
log_MeO_2_E1_0_ngpermgCr  8.3
log_MeO_2_E1_1_ngpermgCr  8.3
log_MeO_4_E1_0_ngpermgCr  8.3
log_MeO_4_E1_1_ngpermgCr  8.3
log_MeO_2_E2_0_ngpermgCr  8.3
log_MeO_2_E2_1_ngpermgCr  8.3
log_MeO_4_E2_0_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3
log_MeO_4_E2_1_ngpermgCr  8.3
log_R_2to16E1_0_ngpermgCr 8.3
log_R_2to16E1_1_ngpermgCr 8.3
log_R_2to4E1_0_ngpermgCr 8.3
log_R_2to4E1_1_ngpermgCr 8.3
log_R_tot_2to4OHE_0_ngpermgCr 8.3
log_R_tot_2to4OHE_1_ngpermgCr 8.3
log_R_tot_2to4MeOE_0_ngpermgCr 8.3
log_R_tot_2to4MeOE_1_ngpermgCr 8.3
log_Total_2OHE_0_ngpermgCr 8.3
log_Total_2OHE_1_ngpermgCr 8.3
log_Total_4OHE_0_ngpermgCr 8.3
log_Total_4OHE_1_ngpermgCr 8.3
log_Total_2MeOE_0_ngpermgCr 8.3
log_Total_2MeOE_1_ngpermgCr 8.3
log_Total_4MeOE_0_ngpermgCr 8.3
log_Total_4MeOE_1_ngpermgCr 8.3;
run;
proc means;
run;

/*PURPOSE: Calculate change in concentration*/
Data EM.D_EM_ngpermgCr_LowSTD;
Set EM.Log_EM_ngpermgCr_LowSTD;
array a[20] E1_0_ngpermgCr E2_0_ngpermgCr E3_0_ngpermgCr
_16_OH_E1_0_ngpermgCr
OH_2_E1_0_ngpermgCr OH_4_E1_0_ngpermgCr OH_2_E2_0_ngpermgCr
OH_4_E2_0_ngpermgCr
MeO_2_E1_0_ngpermgCr MeO_4_E1_0_ngpermgCr
MeO_2_E2_0_ngpermgCr MeO_4_E2_0_ngpermgCr
R_2to16E1_0_ngpermgCr R_2to4E1_0_ngpermgCr
R_tot_2to4OHE_0_ngpermgCr R_tot_2to4MeOE_0_ngpermgCr
Total_2OHE_0_ngpermgCr Total_4OHE_0_ngpermgCr
Total_2MeOE_0_ngpermgCr Total_4MeOE_0_ngpermgCr;
array b[20] E1_1_ngpermgCr E2_1_ngpermgCr E3_1_ngpermgCr
_16_OH_E1_1_ngpermgCr
OH_2_E1_1_ngpermgCr OH_4_E1_1_ngpermgCr
OH_2_E2_1_ngpermgCr
OH_4_E2_1_ngpermgCr
MeO_2_E1_1_ngpermgCr MeO_4_E1_1_ngpermgCr
MeO_2_E2_1_ngpermgCr
MeO_4_E2_1_ngpermgCr
R_2to16E1_1_ngpermgCr R_2to4E1_1_ngpermgCr
R_tot_2to4OHE_1_ngpermgCr R_tot_2to4MeOE_1_ngpermgCr
Total_2OHE_1_ngpermgCr
Total_4OHE_1_ngpermgCr
Total_2MeOE_1_ngpermgCr
Total_4MeOE_1_ngpermgCr;
array c[20] D_E1_ngpermgCr D_E2_ngpermgCr D_E3_ngpermgCr
_16_OH_E1_ngpermgCr
D_OH_2_E1_ngpermgCr
D_OH_4_E1_ngpermgCr
D_OH_2_E2_ngpermgCr
D_OH_4_E2_ngpermgCr
D_MeO_2_E1_ngpermgCr
D_MeO_4_E1_ngpermgCr
D_MeO_2_E2_ngpermgCr
D_MeO_4_E2_ngpermgCr
do j=1 to 20;
   c[j]= b[j]-a[j];
end;
Drop j;

Format D_E1_ngpermgCr 8.3
       D_E2_ngpermgCr 8.3
       D_E3_ngpermgCr 8.3
       D_MeO_2_E1_ngpermgCr 8.3
       D_MeO_4_E1_ngpermgCr 8.3
       D_MeO_2_E2_ngpermgCr 8.3
       D_MeO_4_E2_ngpermgCr 8.3
       D__16_OH_E1_ngpermgCr 8.3
       D_OH_2_E1_ngpermgCr 8.3
       D_OH_4_E1_ngpermgCr 8.3
       D_OH_2_E2_ngpermgCr 8.3
       D_OH_4_E2_ngpermgCr 8.3
       D_R_2to16E1_ngpermgCr 8.3
       D_R_2to4E1_ngpermgCr 8.3
       D_Total_2OHE_ngpermgCr 8.3
       D_Total_4OHE_ngpermgCr 8.3
       D_Total_2MeOE_ngpermgCr 8.3
       D_Total_4MeOE_ngpermgCr 8.3;
RUN;

proc means DATA=EM.D_EM_ngpermgCr_LowSTD;
RUN;

/*PURPOSE: Check the distribution of data, if skewed then need to log transform*/
proc univariate plot data=EM.D_EM_ngpermgCr_LowSTD;
var D_E1_ngpermgCr D_E2_ngpermgCr D_E3_ngpermgCr D__16_OH_E1_ngpermgCr
    D_OH_2_E1_ngpermgCr D_OH_4_E1_ngpermgCr D_OH_2_E2_ngpermgCr
    D_OH_4_E2_ngpermgCr
    D_MeO_2_E1_ngpermgCr D_MeO_4_E1_ngpermgCr D_MeO_2_E2_ngpermgCr
    D_MeO_4_E2_ngpermgCr
    D_R_2to16E1_ngpermgCr D_R_2to4E1_ngpermgCr D_R_tot_2to4OHE_ngpermgCr
    D_R_tot_2to4MeOE_ngpermgCr
    D_Total_2OHE_ngpermgCr D_Total_4OHE_ngpermgCr D_Total_2MeOE_ngpermgCr
    D_Total_4MeOE_ngpermgCr;
/*Distributions are normally distributed so no need to log transform changes from baseline*/
run;

/*PURPOSE: Merge all datasets into one*/
Data Merged_A_LowSTD;
Merge EM.Ratios_EM_ngpermgCr_LowSTD EM.D_EM_ngpermgCr_LowSTD
EM.LOG_EM_ngpermgCr_LowSTD;
RUN;

/*Create a dataset with ID, trt, age_strata and bmi_strata to merge to EM.EM_318*/
Data Merged_B_LowSTD;
Set WISER.Table1;
keep id trt age_strata bmi_strata bmi_class bmi_over25;
run;

Data Merged_B_LowSTD;
Set Merged_B_LowSTD;
If ID='2622' then DELETE;
if ID='2601' then DELETE;
RUN;

/*PURPOSE: Add to merged data set id, trt and age & bmi strata*/
Data EM.EM_Merged_ngpermgCr_LowSTD;
Merge Merged_B_LowSTD Merged_A_LowSTD;
RUN;

/*PURPOSE: Calculate EM ratios (nmol EM/day)*/
Data EM.Ratios_EM_nmolperday_LowSTD;
Set EM.Ratios_EM_nmolperday_LowSTD;

R_2to16E1_0_nmolperday = OH_2_E1_0_nmolperday/16_OH_E1_0_nmolperday;
R_2to16E1_1_nmolperday = OH_2_E1_1_nmolperday/16_OH_E1_1_nmolperday;
R_2to4E1_0_nmolperday = OH_2_E1_0_nmolperday/OH_4_E1_0_nmolperday;
R_2to4E1_1_nmolperday = OH_2_E1_1_nmolperday/OH_4_E1_1_nmolperday;
Total_2OHE_0_nmolperday = (OH_2_E1_0_nmolperday +
                          OH_2_E2_0_nmolperday);
Total_2OHE_1_nmolperday = (OH_2_E1_1_nmolperday +
                          OH_2_E2_1_nmolperday);
Total_4OHE_0_nmolperday = (OH_4_E1_0_nmolperday +
                          OH_4_E2_0_nmolperday);
Total_4OHE_1_nmolperday = (OH_4_E1_1_nmolperday +
                          OH_4_E2_1_nmolperday);
Total_2MeOE_0_nmolperday = (MeO_2_E1_0_nmolperday +
                           MeO_2_E2_0_nmolperday);
Total_2MeOE_1_nmolperday = (MeO_2_E1_1_nmolperday +
                           MeO_2_E2_1_nmolperday);
Total_4MeOE_0_nmolperday = (MeO_4_E1_0_nmolperday +
                           MeO_4_E2_0_nmolperday);
Total_4MeOE_1_nmolperday = (MeO_4_E1_1_nmolperday +
                           MeO_4_E2_1_nmolperday);
R_tot_2to4OHE_0_nmolperday = (Total_2OHE_0_nmolperday/Total_4OHE_0_nmolperday);
R_tot_2to4OHE_1_nmolperday = (Total_2OHE_1_nmolperday/Total_4OHE_1_nmolperday);
R_tot_2to4MeOE_0_nmolperday = (Total_2MeOE_0_nmolperday/Total_4MeOE_0_nmolperday);
R_tot_2to4MeOE_1_nmolperday = (Total_2MeOE_1_nmolperday/Total_4MeOE_1_nmolperday);

FORMAT
/*RESULT: All hormones are right-skewed (max/min >10) so need to log transform*/

Data EM.Log_EM_nmolperday_LowSTD;
Set EM.Ratios_EM_nmolperday_LowSTD;

log_E1_0_nmolperday                = log(E1_0_nmolperday);  
log_E1_1_nmolperday                = log(E1_1_nmolperday);  
log_E2_0_nmolperday                = log(E2_0_nmolperday);  
log_E2_1_nmolperday                = log(E2_1_nmolperday);  
log_E3_0_nmolperday                = log(E3_0_nmolperday);  
log_E3_1_nmolperday                = log(E3_1_nmolperday);  
log__16_OH_E1_0_nmolperday         = log(_16_OH_E1_0_nmolperday); 
log__16_OH_E1_1_nmolperday         = log(_16_OH_E1_1_nmolperday); 
log_OH_2_E1_0_nmolperday           = log(OH_2_E1_0_nmolperday);  
log_OH_2_E1_1_nmolperday           = log(OH_2_E1_1_nmolperday);  
log_OH_4_E1_0_nmolperday           = log(OH_4_E1_0_nmolperday);  
log_OH_4_E1_1_nmolperday           = log(OH_4_E1_1_nmolperday);  
log_OH_2_E2_0_nmolperday           = log(OH_2_E2_0_nmolperday);  
log_OH_2_E2_1_nmolperday           = log(OH_2_E2_1_nmolperday);  
log_OH_4_E2_0_nmolperday           = log(OH_4_E2_0_nmolperday);  
log_OH_4_E2_1_nmolperday           = log(OH_4_E2_1_nmolperday);  
log_MeO_2_E1_0_nmolperday          = log(MeO_2_E1_0_nmolperday);  
log_MeO_2_E1_1_nmolperday          = log(MeO_2_E1_1_nmolperday);  
log_MeO_4_E1_0_nmolperday          = log(MeO_4_E1_0_nmolperday);  
log_MeO_4_E1_1_nmolperday          = log(MeO_4_E1_1_nmolperday);  
log_MeO_2_E2_0_nmolperday          = log(MeO_2_E2_0_nmolperday);  
log_MeO_2_E2_1_nmolperday          = log(MeO_2_E2_1_nmolperday);  
log_MeO_4_E2_0_nmolperday          = log(MeO_4_E2_0_nmolperday);  
log_MeO_4_E2_1_nmolperday          = log(MeO_4_E2_1_nmolperday);  
log_R_2to16E1_0_nmolperday         = log(R_2to16E1_0_nmolperday);  
log_R_2to16E1_1_nmolperday         = log(R_2to16E1_1_nmolperday);  

log_R_2to4E1_0_nmolperday = log(R_2to4E1_0_nmolperday);
log_R_2to4E1_1_nmolperday = log(R_2to4E1_1_nmolperday);
log_R_tot_2to4OHE_0_nmolperday = log(R_tot_2to4OHE_0_nmolperday);
log_R_tot_2to4OHE_1_nmolperday = log(R_tot_2to4OHE_1_nmolperday);
log_R_tot_2to4MeOE_0_nmolperday = log(R_tot_2to4MeOE_0_nmolperday);
log_R_tot_2to4MeOE_1_nmolperday = log(R_tot_2to4MeOE_1_nmolperday);
log_Total_2OHE_0_nmolperday = log(Total_2OHE_0_nmolperday);
log_Total_2OHE_1_nmolperday = log(Total_2OHE_1_nmolperday);
log_Total_4OHE_0_nmolperday = log(Total_4OHE_0_nmolperday);
log_Total_4OHE_1_nmolperday = log(Total_4OHE_1_nmolperday);
log_Total_2MeOE_0_nmolperday = log(Total_2MeOE_0_nmolperday);
log_Total_2MeOE_1_nmolperday = log(Total_2MeOE_1_nmolperday);
log_Total_4MeOE_0_nmolperday = log(Total_4MeOE_0_nmolperday);
log_Total_4MeOE_1_nmolperday = log(Total_4MeOE_1_nmolperday);

Format
log_E1_0_nmolperday 8.3
log_E1_1_nmolperday 8.3
log_E2_0_nmolperday 8.3
log_E2_1_nmolperday 8.3
log_E3_0_nmolperday 8.3
log_E3_1_nmolperday 8.3
log__16_OH_E1_0_nmolperday 8.3
log__16_OH_E1_1_nmolperday 8.3
log_OH_2_E1_0_nmolperday 8.3
log_OH_2_E1_1_nmolperday 8.3
log_OH_4_E1_0_nmolperday 8.3
log_OH_4_E1_1_nmolperday 8.3
log_OH_2_E2_0_nmolperday 8.3
log_OH_2_E2_1_nmolperday 8.3
log_OH_4_E2_0_nmolperday 8.3
log_OH_4_E2_1_nmolperday 8.3
log_MeO_2_E1_0_nmolperday 8.3
log_MeO_2_E1_1_nmolperday 8.3
log_MeO_4_E1_0_nmolperday 8.3
log_MeO_4_E1_1_nmolperday 8.3
log_MeO_2_E2_0_nmolperday 8.3
log_MeO_2_E2_1_nmolperday 8.3
log_MeO_4_E2_0_nmolperday 8.3
log_MeO_4_E2_1_nmolperday 8.3
log_R_2to16E1_0_nmolperday 8.3
log_R_2to16E1_1_nmolperday 8.3
log_R_2to4E1_0_nmolperday 8.3
log_R_2to4E1_1_nmolperday 8.3
log_R_tot_2to4OHE_0_nmolperday 8.3
log_R_tot_2to4OHE_1_nmolperday 8.3
log_R_tot_2to4MeOE_0_nmolperday 8.3
log_R_tot_2to4MeOE_1_nmolperday 8.3
log_Total_2OHE_0_nmolperday 8.3
log_Total_2OHE_1_nmolperday 8.3
log_Total_4OHE_0_nmolperday 8.3
log_Total_4OHE_1_nmolperday 8.3
log_Total_2MeOE_0_nmolperday 8.3
/*PURPOSE: Calculate change in concentration*/
Data EM.D_EM_nmolperday_LowSTD;
Set EM.Log_EM_nmolperday_LowSTD;
array a[20] E1_0_nmolperday E2_0_nmolperday E3_0_nmolperday 
_16_OH_E1_0_nmolperday 
OH_2_E1_0_nmolperday OH_2_E2_0_nmolperday OH_4_E1_0_nmolperday 
OH_4_E2_0_nmolperday 
MeO_2_E1_0_nmolperday MeO_2_E2_0_nmolperday 
MeO_4_E1_0_nmolperday MeO_4_E2_0_nmolperday 
R_2to16E1_0_nmolperday R_2to4E1_0_nmolperday 
R_tot_2to4OHE_0_nmolperday R_tot_2to4MeOE_0_nmolperday 
Total_2OHE_0_nmolperday Total_4OHE_0_nmolperday 
Total_2MeOE_0_nmolperday Total_4MeOE_0_nmolperday;
array b[20] E1_1_nmolperday E2_1_nmolperday E3_1_nmolperday 
_16_OH_E1_1_nmolperday 
OH_2_E1_1_nmolperday OH_2_E2_1_nmolperday OH_4_E1_1_nmolperday 
OH_4_E2_1_nmolperday 
MeO_2_E1_1_nmolperday MeO_2_E2_1_nmolperday 
MeO_4_E1_1_nmolperday MeO_4_E2_1_nmolperday 
R_2to16E1_1_nmolperday R_2to4E1_1_nmolperday 
R_tot_2to4OHE_1_nmolperday R_tot_2to4MeOE_1_nmolperday 
Total_2OHE_1_nmolperday Total_4OHE_1_nmolperday 
Total_2MeOE_1_nmolperday Total_4MeOE_1_nmolperday;
array c[20] D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday 
_D_16_OH_E1_nmolperday 
D_OH_2_E1_nmolperday D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday 
D_OH_4_E2_nmolperday 
D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday 
D_MeO_4_E1_nmolperday D_MeO_4_E2_nmolperday 
D_R_2to16E1_nmolperday D_R_2to4E1_nmolperday 
D_R_tot_2to4OHE_nmolperday D_R_tot_2to4MeOE_nmolperday 
D_Total_2OHE_nmolperday D_Total_4OHE_nmolperday 
D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday;
do j=1 to 20;
c[j]= b[j]-a[j];
end;
Drop j;
Format D_E1_nmolperday 8.3 
D_E2_nmolperday 8.3 
D_E3_nmolperday 8.3
D_16_OH_E1_nmolperday 8.3
D_OH_2_E1_nmolperday 8.3
D_OH_2_E2_nmolperday 8.3
D_OH_4_E1_nmolperday 8.3
D_OH_4_E2_nmolperday 8.3
D_MeO_2_E1_nmolperday 8.3
D_MeO_2_E2_nmolperday 8.3
D_MeO_4_E1_nmolperday 8.3
D_MeO_4_E2_nmolperday 8.3
D_R_2to16E1_nmolperday 8.3
D_R_2to4E1_nmolperday 8.3
D_Total_2OHE_nmolperday 8.3
D_Total_4OHE_nmolperday 8.3
D_Total_2MeOE_nmolperday 8.3
D_Total_4MeOE_nmolperday 8.3
D_R_tot_2to4OHE_nmolperday 8.3
D_R_tot_2to4MeOE_nmolperday 8.3;

RUN;

**PURPOSE:** Check the distribution of data, if skewed then need to log transform*/

**proc univariate plot data=EM.D_EM_nmolperday_LowSTD;**

**var**

D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday D_16_OH_E1_nmolperday
D_OH_2_E1_nmolperday D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday
D_OH_4_E2_nmolperday D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_MeO_4_E1_nmolperday
D_MeO_4_E2_nmolperday D_R_2to16E1_nmolperday D_R_2to4E1_nmolperday
D_R_tot_2to4OHE_nmolperday D_R_tot_2to4MeOE_nmolperday
D_Total_2OHE_nmolperday D_Total_4OHE_nmolperday D_Total_2MeOE_nmolperday
D_Total_4MeOE_nmolperday;

/*Distributions are normally distributed so no need to log transform changes from baseline*/
run;

/*PURPOSE:** Merge all datasets into one*/

**Data** Merged_A_LowSTD;

**Merge**

EM.Ratios_EM_nmolperday_LowSTD EM.D_EM_nmolperday_LowSTD EM.LOG_EM_nmolperday_LowSTD;

RUN;

/*Create a dataset with ID, trt, age_strata and bmi_strata to merge to EM.EM_318*/

**Data** Merged_B_LowSTD;

**Set** WISER.Table1;

**keep** id trt age_strata bmi_strata bmi_class bmi_over25 lean_mass_0;

run;

**Data** Merged_B_LowSTD;

**Set** Merged_B_LowSTD;

If ID='2622' then DELETE;
if ID='2601' then DELETE;

RUN;
/*PURPOSE: Add to merged EM data, id, trt and age & bmi strata, lean_mass_0*/
Data EM.EM_Merged_nmolperday_LowSTD;
Merge Merged_B_LowSTD Merged_A_LowSTD;
RUN;

/*PURPOSE: To investigate baseline associations between EM and suggested covariates based on lit review*/
Data EM.BaselineAssoc_ngpermgcr;
Merge WISER.Demographics_319 WISER.Bodycomp_319 Wiser.Bodyweights_319
WISER.Menarche_310 WISER.Familyhistory_251
WISER.FoodRecords_312 WISER.Fitness_319 WISER.SRPA_319 EM.EM_Merged_ngpermgcr;
by ID;
If ID= '2601' then DELETE;
If ID= '2622' then DELETE;
KEEP ID trt age age_strata bmi_strata bmi_class
  Weight_0 D_weight BMI_0 D_BMI fat_mass_0 D_fat_mass lean_mass_0 D_lean_mass
  Menarche_age
  Kcal_0 Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 Caff_mg_0 Alc_g_0
  Mets_0 total_mets_week_0
  log_E1_0_ngpermgCr log_E2_0_ngpermgCr log_E3_0_ngpermgCr
  log__16_OH_E1_0_ngpermgCr
  log_OH_2_E1_0_ngpermgCr log_OH_4_E1_0_ngpermgCr log_MeO_2_E1_0_ngpermgCr
  log_MeO_2_E2_0_ngpermgCr
  log_R_2to16_E1_0_ngpermgCr log_R_2to4_E1_0_ngpermgCr
  D_E1_ngpermgCr D_E2_ngpermgCr D_E3_ngpermgCr D__16_OH_E1_ngpermgCr
  D_OH_2_E1_ngpermgCr D_OH_4_E1_ngpermgCr
  D_MeO_2_E1_ngpermgCr D_MeO_2_E2_ngpermgCr
  D_R_2to16_E1_ngpermgCr D_R_2to4_E1_ngpermgCr
RUN;

PROC means DATA=EM.BASELINEassoc_ngpermgcr;
/*since caff_mg_0 and alc_g_0 have a min value=0 these need to be log transformed*/
run;

Data EM.BaselineAssoc_ngpermgcr;
Set EM.BaselineAssoc_ngpermgcr;
log_caff = log(caff_mg_0 + 1.0);
log_alc= log(alc_g_0 + 1.0);
DROP caff_mg_0 alc_g_0;
run;

PROC means DATA=EM.BASELINEassoc_ngpermgcr n nmiss max min;
/*since caff_mg_0 and alc_g_0 have a min value=0 these need to be log transformed*/
run;

Proc Corr Data=EM.BaselineAssoc_ngpermgCr;
var log_E1_0_ngpermgCr log_E2_0_ngpermgCr log_E3_0_ngpermgCr
log_OH_2_E1_0_ngpermgCr log_OH_4_E1_0_ngpermgCr log_MeO_2_E1_0_ngpermgCr
log_MeO_2_E2_0_ngpermgCr log_R_2to16E1_0_ngpermgCr log_R_2to4E1_0_ngpermgCr;
with age Weight_0 D_weight BMI_0 D_BMI fat_mass_0 D_fat_mass lean_mass_0 D_lean_mass
Menarche_age Kcal_0 Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 log_Caff log_Alc
Mets_0 total_mets_week_0;
run;

Proc Reg data=em.baselineassoc_ngpermgcr;
model /*log_OH_2_E1_0_ngpermgCr log_OH_4_E1_0_ngpermgCr*/ log_R_2to16E1_0_ngpermgCr=
age Weight_0 BMI_0 fat_mass_0 lean_mass_0
Menarche_age Kcal_0 Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 log_Caff log_Alc
Mets_0 total_mets_week_0/VIF;
plot cookd. * obs.;
output out=c cookd=cook_dist;
run;
proc print data=c;
where (cook_dist> 0.1);
run;

Proc Corr data=em.baselineassoc_ngpermgcr;
var weight_0 bmi_0 fat_mass_0 lean_mass_0 kcal_0 fat_g_0 carb_g_0;
run;

Proc Insight data=em.baselineassoc_ngpermgcr;
/*scatter weight_0 * fat_mass_0 bmi_0 lean_mass_0;*/remove weight*/
sscatter bmi_0 * fat_mass_0 lean_mass_0; /*remove bmi*/
/*scatter fat_mass_0 * lean_mass_0;*/no colinearity
scatter kcal_0 * fat_g_0 carb_g_0; /*remove kcal_0*/
run;
/*predictors are highly correlated so remove one (bmi_0)*/
quit;

Proc Reg data=em.baselineassoc_ngpermgcr;
model /*log_OH_2_E1_0_ngpermgCr log_OH_4_E1_0_ngpermgCr*/ log_R_2to16E1_0_ngpermgCr =
age fat_mass_0 lean_mass_0
Menarche_age Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 log_Caff log_Alc
Mets_0 total_mets_week_0/ selection=cp;
run;
plot student. * predicted.;
output out=c predicted=yhat student=resid;
run;

Proc corr data=em.baselineassoc_ngpermgcr;
var log_OH_2_E1_0_ngpermgCr;
with total_mets_week_0 Fib_g_0;
run;
**Proc Reg data**=em.baselineassoc_ngpermgcr;
**model** log\_OH\_2\_E1\_0\_ngpermgCr = Fib\_g\_0; /*r squared= 0.03*/
**run**;

**Proc corr data**=em.baselineassoc_ngpermgcr;
**var** log\_16\_OH\_E1\_0\_ngpermgCr;
**with** Fat\_mass\_0 Lean\_mass\_0 Carb\_g\_0 Fib\_g\_0 log\_alc Mets\_0;
**run**;

**Proc Reg data**=em.baselineassoc_ngpermgcr;
**model** log\_16\_OH\_E1\_0\_ngpermgCr = log\_alc Mets\_0; /*r squared= 0.04*/
**run**;

**Proc corr data**=em.baselineassoc_ngpermgcr;
**var** log\_R\_2to16E1\_0\_ngpermgCr;
**with** Fat\_mass\_0 Fat\_g\_0 Prot\_g\_0 Carb\_g\_0 Fib\_g\_0 log\_alc Mets\_0 total\_mets\_week\_0;
**run**;

**Proc Reg data**=em.baselineassoc_ngpermgcr;
**model** log\_R\_2to16E1\_0\_ngpermgCr = log\_alc total\_Mets\_week\_0; /*r squared= 0.04*/
**run**;

/*PURPOSE: To investigate baseline associations between EM and suggested covariates based on lit review inverse relationship with age, age at menarche*/

**Proc Sort Data**=EM.EM\_Merged\_nmolperday\_LowSTD;
**By** id;
**run**;

**Data** EM.BaselineAssoc\_nmolperday;
**Merge** WISER.Demographics\_319 WISER.Bodycomp\_319 Wiser.Bodyweights\_319
WISER.Menarche\_310 WISER.Familyhistory\_251
WISER.FoodRecords\_312 WISER.Fitness\_319 WISER.SRPA\_319
EM.EM\_Merged\_nmolperday\_LowSTD;
**by** ID;
**If** ID='2601' then DELETE;
**If** ID='2622' then DELETE;
**KEEP** ID trt age age\_strata bmi\_strata
Weight\_0 D\_weight BMI\_0 D\_BMI fat\_mass\_0 D\_fat\_mass lean\_mass\_0 D\_lean\_mass
Menarche\_age
Kcal\_0 Fat\_g\_0 Carb\_g\_0 Prot\_g\_0 Fib\_g\_0 Caff\_mg\_0 Alc\_g\_0
Mets\_0 total\_mets\_week\_0
log\_E1\_0\_nmolperday log\_E2\_0\_nmolperday log\_E3\_0\_nmolperday
log\_16\_OH\_E1\_0\_nmolperday
log\_OH\_2\_E1\_0\_nmolperday log\_OH\_4\_E1\_0\_nmolperday log\_MeO\_2\_E1\_0\_nmolperday
log\_MeO\_2\_E2\_0\_nmolperday
log\_R\_2to16E1\_0\_nmolperday log\_R\_2to4E1\_0\_nmolperday;
**RUN**;

**PROC means DATA**=EM.BASELINEassoc\_nmolperday;
/*since caff\_mg\_0 and alc\_g\_0 have a min value=0 these need to be log transformed*/
run;

**Data** EM.BaselineAssoc_nmolperday;
**Set** EM.BaselineAssoc_nmolperday;

\[
\begin{align*}
\text{log}_e \text{caff} &= \log(\text{caff}_0 + 1.0) ; \\
\text{log}_e \text{alc} &= \log(\text{alc}_0 + 1.0) ;
\end{align*}
\]

\text{DROP} \text{caff}_0 \text{alc}_0 ;
run;

**PROC means** DATA=EM.BASELINEassoc_nmolperday n nmiss max min; /*since caff_mg_0 and alc_g_0 have a min value=0 these need to be log transformed*/
run;

/* Purpose: to check any correlation between EM and body composition measures*/
**Proc Corr Data**=EM.BaselineAssoc_nmolperday;

\text{var} \text{age} \text{Weight}_0 \text{BMI}_0 \text{BMI} \text{fat}_mass_0 \text{D} \text{fat_mass} \text{lean_mass}_0 \text{D} \text{lean_mass} \text{Menarche_age} \\
\text{Kcal}_0 \text{Fat}_g_0 \text{Carb}_g_0 \text{Prot}_g_0 \text{Fib}_g_0 \text{log}_e \text{Caff} \text{log}_e \text{Alc} \\
\text{Mets}_0 \text{total_mets}_week_0 ;

\text{with} /*\text{log}_e \text{E1}_0 \text{nmolperday} \text{log}_e \text{E2}_0 \text{nmolperday} \text{log}_e \text{E3}_0 \text{nmolperday} \\
\text{log}_e \text{16-OH_E1}_0 \text{nmolperday} \\
\text{log}_e \text{OH}_2 \text{E1}_0 \text{nmolperday} /*\text{log}_e \text{OH}_4 \text{E1}_0 \text{nmolperday} \text{log}_e \text{MeO}_2 \text{E1}_0 \text{nmolperday} \\
\text{log}_e \text{R}_2\text{to16E1}_0 \text{nmolperday} /*\text{log}_e \text{R}_2\text{to4E1}_0 \text{nmolperday}*/;
run; /*2OHE1 r=0.216 p=0.0001 with leanmass*/

**Proc GLM data**=em.baselineassoc_nmolperday;

\text{class} \text{trt} \text{age_strata} \text{bmi_strata} ;

\text{model} \text{log}_e \text{OH}_2 \text{E1}_0 \text{nmolperday} = \\
\text{age} \\
\text{lean_mass}_0 \\
\text{Kcal}_0 \text{Fat}_g_0 \text{Carb}_g_0 \text{Fib}_g_0 \\
/\text{ss3};
run;

**Proc Reg data**=em.baselineassoc_nmolperday;

\text{model} \text{log}_e \text{E2}_0 \text{nmolperday} = \text{age} \\
\text{Weight}_0 \text{BMI}_0 \text{fat_mass}_0 \text{lean_mass}_0 \\
\text{Kcal}_0 \text{Carb}_g_0 \text{log}_e \text{Caff}/ \text{VIF};

\text{plot} \text{cookd}. * \text{obs.} ;
\text{output out=\text{\text{c}} cookd=cook_dist};
run;
**proc print data**=\text{\text{c}};
\text{where} (\text{\text{cook_dist}} > 0.10) ;
run;

**Proc Corr data**=em.baselineassoc_nmolperday;

\text{var} \text{weight}_0 \text{bmi}_0 \text{fat_mass}_0 \text{lean_mass}_0 \text{kcal}_0 \text{fat}_g_0 \text{carb}_g_0 ;
run;
**Proc Insight** data=em.baselineassoc_nmolperday;
/*scatter weight_0 * fat_mass_0 bmi_0 lean_mass_0;*/remove weight*/
/*scatter bmi_0 * fat_mass_0 lean_mass_0; /*remove bmi*/
scatter fat_mass_0 * lean_mass_0; /*no colinearity*/
run;
/*predictors are highly correlated so remove one (bmi_0)*/
quit;

**Proc Reg** data=em.baselineassoc_nmolperday;
model /*log_E2_0_nmolperday log_E1_0_nmolperday log_R_2to16E1_0_nmolperday*/
log_OH_2_E1_0_nmolperday
   = age
   Weight_0 D_weight BMI_0 D_BMI fat_mass_0 D_fat_mass lean_mass_0 D_lean_mass
   Menarche_age
   Kcal_0 Fat_g_0 Carb_g_0 Prot_g_0 Fib_g_0 log_Caff log_Alc
   Mets_0 total_mets_week_0
   / selection=cp;
run;
plot student.* predicted.;
output out=c predicted=yhat student=resid;
run;

/*Table 1. Baseline characteristics of Randomized participants (n=318) ID 2622 Did not complete CV8 due to travel*/
**Proc Sort** Data=WISER.SRPA_319;
BY ID;
RUN;
**Data** WISER.EM_Table1;
Merge WISER.Demographics_319 WISER.Bodycomp_319 WISER.Menarche_310
WISER.Familyhistory_251 WISER.FoodRecords_312
WISER.Fitness_319 wiser.srpa_319;
By id;
KEEP
ID trt age age_strata Ht_CV1 Wt_CV1 fat_mass_0 Percent_bodyfat_0 lean_mass_0 BMI BMI_class
   BMI_class4
   race Hispanic EDU MarStat status
   Menarche_age BC_history BCuse Parity
   Kcal_0 FatCals_0 Fib_g_0 Caff_mg_0 Alc_g_0 total_mets_week_0
   Mets_0
;
run;
**Data** WISER.EM_Table1;
Set WISER.EM_Table1;
IF ID = '2622' THEN DELETE;
If EDU = 'College/University degree' then Education = 'Beyond HS';
If EDU = 'Graduate or Professional degree' then Education = 'Beyond HS';
If EDU = 'Some college (less than 4 years)' then Education = 'Beyond HS';
If EDU = 'Vocational training (beyond high school)' then Education = 'Beyond HS';
If EDU = 'Completed high school diploma (or GED)' then Education = 'HS or less';
IF EDU = 'Less than high school diploma' then Education = 'HS or less';
RUN;
**Proc Ttest** Data=wiser.EM_Table1 ci=none;
class trt;
var age Wt_CV1 BMI fat_mass_0 Percent_bodyfat_0 lean_mass_0 Kcal_0 Alc_g_0 Mets_0 total_mets_week_0 menarche_age;
run;

**Proc Freq** Data=wiser.EM_Table1;
tables trt*(BMI_class4 race Hispanic Education menarche_age MarStat status BC_history BCuse Parity BMI_class age_strata)
/ nocol chisq;
run;

/*Table 2. Baseline EM characteristics of Randomized participants (n=318)
ID 2622 Did not complete CV8 due to travel*/
**Proc sort** Data=EM.EM_Merged_ngpermgCr_LowSTD;
by trt;
run;

**Proc Ttest** Data=EM.EM_Merged_ngpermgCr_LowSTD ci=none;
class trt;
var E1_0_ngpermgCr E2_0_ngpermgCr E3_0_ngpermgCr _16_OH_E1_0_ngpermgCr OH_2_E1_0_ngpermgCr OH_4_E1_0_ngpermgCr OH_2_E2_0_ngpermgCr OH_4_E2_0_ngpermgCr MeO_2_E1_0_ngpermgCr MeO_4_E1_0_ngpermgCr MeO_2_E2_0_ngpermgCr MeO_4_E2_0_ngpermgCr R_2to16E1_0_ngpermgCr R_2to4E1_0_ngpermgCr;
/*No differences in baseline levels*/
run;

**proc means** mean median Q1 Q3 data=EM.EM_Merged_ngpermgCr_LowSTD;
var E1_0_ngpermgCr E2_0_ngpermgCr E3_0_ngpermgCr _16_OH_E1_0_ngpermgCr OH_2_E1_0_ngpermgCr OH_4_E1_0_ngpermgCr OH_2_E2_0_ngpermgCr OH_4_E2_0_ngpermgCr MeO_2_E1_0_ngpermgCr MeO_4_E1_0_ngpermgCr MeO_2_E2_0_ngpermgCr MeO_4_E2_0_ngpermgCr R_2to16E1_0_ngpermgCr R_2to4E1_0_ngpermgCr;
by trt;
run;

/*PURPOSE; Generate Table 2. Distribution of Baseline urinary levels of wiser participants by group
(n=318)
ID 2622 Did not complete CV8 due to travel*/

/*Calculate baseline median, 10th and 90th percentile levels per group*/
**Proc sort** Data=EM.EM_Merged_nmolperday_LowSTD;
by trt;
run;
**proc means** P50 P10 P90 data=EM.EM_Merged_nmolperday_LowSTD;
var E1_0_nmolperday E2_0_nmolperday E3_0_nmolperday _16_OH_E1_0_nmolperday OH_2_E1_0_nmolperday OH_4_E1_0_nmolperday OH_2_E2_0_nmolperday OH_4_E2_0_nmolperday
MeO_2_E1_0 nmol/permday MeO_2_E2_0 nmol/permday MeO_4_E1_0 nmol/permday MeO_4_E2_0 nmol/permday R_2to16E1_0 nmol/permday R_2to4E1_0 nmol/permday;
by trt;
run;

/*Determine if there are significant differences at baseline in urinary levels of EM
Given non-normality, comparisons must be made using log transformed values*/

Proc Ttest Data=EM.EM_Merged_nmol/permday_LowSTD ci=none;
class trt;
var log_E1_0 nmol/permday log_E2_0 nmol/permday log_E3_0 nmol/permday log_16_OH_E1_0 nmol/permday log_OH_2_E1_0 nmol/permday log_OH_2_E2_0 nmol/permday log_OH_4_E1_0 nmol/permday log_OH_4_E2_0 nmol/permday log_MeO_2_E1_0 nmol/permday log_MeO_2_E2_0 nmol/permday log_MeO_4_E1_0 nmol/permday log_MeO_4_E2_0 nmol/permday log_R_2to16E1_0 nmol/permday log_R_2to4E1_0 nmol/permday;
/*No differences in baseline levels*/
run;

/* PURPOSE: To conduct baseline comparisons (ng/mg Cr) using a full model*/

Proc GLM data=EM.EM_Merged_ng/permCr_LowSTD;
class age_strata bmi_strata trt;
model log_E1_0 ng/permCr log_E2_0 ng/permCr log_E3_0 ng/permCr log_16_OH_E1_0 ng/permCr
      log_OH_2_E1_0 ng/permCr log_OH_2_E2_0 ng/permCr log_OH_4_E1_0 ng/permCr
      log_OH_4_E2_0 ng/permCr log_MeO_2_E1_0 ng/permCr log_MeO_2_E2_0 ng/permCr
      log_MeO_4_E1_0 ng/permCr log_MeO_4_E2_0 ng/permCr log_R_2to16E1_0 ng/permCr
      log_R_2to4E1_0 ng/permCr log_R_tot_2to4OHE_0 ng/permCr log_R_tot_2to4MeOE_0 ng/permCr
      log_Total_2OHE_0 ng/permCr log_Total_4OHE_0 ng/permCr log_Total_2MeOE_0 ng/permCr
      log_Total_4MeOE_0 ng/permCr = age_strata | BMI_strata | trt/ ss3;
run;
/*SIGNIFICANT TRT INTERACTIONS*/
quit;

/*Per Will Thomas, these variables should be run with a simplified model. Keeping the trt interaction means
we should present the comparisons in each of 6 age-bmi strata separately*/

/*PURPOSE: Conduct baseline comparisons (ng/mg Cr) using a reduced model for all variables*/

Proc GLM Data=EM.EM_Merged_ng/permCr_LowSTD;
class age_strata bmi_strata trt;
model log_E1_0 ng/permCr log_E2_0 ng/permCr log_E3_0 ng/permCr log_16_OH_E1_0 ng/permCr
      log_OH_2_E1_0 ng/permCr log_OH_2_E2_0 ng/permCr log_OH_4_E1_0 ng/permCr
      log_OH_4_E2_0 ng/permCr log_MeO_2_E1_0 ng/permCr log_MeO_2_E2_0 ng/permCr
      log_MeO_4_E1_0 ng/permCr log_MeO_4_E2_0 ng/permCr log_R_2to16E1_0 ng/permCr log_R_2to4E1_0 ng/permCr
log_R_tot_2to4OHE_0_ngpermgCr log_R_tot_2to4MeOE_0_ngpermgCr log_Total_2OHE_0_ngpermgCr log_Total_4OHE_0_ngpermgCr log_Total_2MeOE_0_ngpermgCr log_Total_4MeOE_0_ngpermgCr
= trt age_strata BMI_strata ss3;
lsmeans trt / CL pdiff;
ODS output LSMeanCL=logCI_0;
run;
/*Significant difference at baseline in log(2/16OHE1) p=0.044 so will need to adjust change from baseline comparisons*/
quit;

Data a;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCI);
right_CI= exp(UpperCI);
drop lsmean LowerCL UpperCL;
run;
proc print data =a;
run;

/* PURPOSE: To conduct changes from baseline comparisons (ng/mg Cr)using a full model.
Since there were significant differences at baseline in 2/16 ratio, include baseline term in the model*/
Proc GLM Data =EM.EM_Merged_ngpermgCr.LowSTD;
class age_strata bmi_strata trt;
model D_E1_ngpermgCr D_E2_ngpermgCr D_E3_ngpermgCr D_16_OH_E1_ngpermgCr
D_OH_2_E1_ngpermgCr D_OH_2_E2_ngpermgCr D_OH_4_E1_ngpermgCr
D_OH_4_E2_ngpermgCr
D_MeO_2_E1_ngpermgCr D_MeO_2_E2_ngpermgCr D_MeO_4_E1_ngpermgCr
D_MeO_4_E2_ngpermgCr
D_R_2to4E1_ngpermgCr
D_R_tot_2to4OHE_ngpermgCr D_R_tot_2to4MeOE_ngpermgCr
D_Total_2OHE_ngpermgCr D_Total_4OHE_ngpermgCr D_Total_2MeOE_ngpermgCr
D_Total_4MeOE_ngpermgCr
= age_strata BMI_strata trt / ss3;
lsmeans trt / stderr pdiff;
run;
quit;

Proc GLM Data =EM.EM_Merged_ngpermgCr.LowSTD;
class age_strata bmi_strata trt;
model D_R_2to16E1_ngpermgCr = log_R_2to16E1_0_ngpermgCr age_strata BMI_strata trt / ss3;
lsmeans trt / stderr pdiff;
run;
quit;

/* PURPOSE: To conduct changes from baseline comparisons (ng/mg Cr)using a reduced model.
Since there were significant differences at baseline in 2/16 ratio, include baseline term in the model*/
Proc GLM Data =EM.EM_Merged_ngpermgCr.LowSTD;
class age_strata bmi_strata trt;
model D_E1_ngpermgCr D_E2_ngpermgCr D_E3_ngpermgCr D_16_OH_E1_ngpermgCr
D_OH_2_E1_ngpermgCr D_OH_2_E2_ngpermgCr D_OH_4_E1_ngpermgCr
D_OH_4_E2_ngpermgCr
D_MeO_2_E1_ngpermgCr D_MeO_2_E2_ngpermgCr D_MeO_4_E1_ngpermgCr
D_MeO_4_E2_ngpermgCr
D_R_2to4E1_ngpermgCr
D_R_tot_2to4OH_E1_ngpermgCr D_R_tot_2to4MeOE_ngpermgCr
D_Total_2OHE_ngpermgCr D_Total_4OHE_ngpermgCr D_Total_2MeOE_ngpermgCr
D_Total_4MeOE_ngpermgCr

lsmeans trt / stderr pdiff CL;
run;

/*Significant within change in controls in E2 p=0.028*/
quit;

Proc GLM Data =EM.EM_Merged_ngpermgCr_LowSTD;
class age_strata bmi_strata trt;
model D_R_2to16E1_ngpermgCr = trt log_R_2to16E1_0_ngpermgCr age_strata / bmi_strata / ss3;
lsmeans trt / stderr pdiff CL;
run;

/*Exercisers significantly increase their ratio (p=0.034) which was different from the controls (p=0.033) Significant change between groups in 2/16 p=0.045*/
quit;

/* PURPOSE: To conduct baseline comparisons (nmol/day) using a full model*/

Proc GLM data=EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model log_E1_0_nmolperday log_E2_0_nmolperday log_E3_0_nmolperday
log_16_OH_E1_0_nmolperday
log_OH_2_E1_0_nmolperday log_OH_2_E2_0_nmolperday log_OH_4_E1_0_nmolperday
log_OH_4_E2_0_nmolperday
log_MeO_2_E1_0_nmolperday log_MeO_2_E2_0_nmolperday log_MeO_4_E1_0_nmolperday
log_MeO_4_E2_0_nmolperday
log_R_2to16E1_0_nmolperday log_R_2to4E1_0_nmolperday
log_R_tot_2to4MeOE_0_nmolperday log_R_tot_2to4OH_E0_nmolperday
log_Total_2OHE_0_nmolperday log_Total_4OHE_0_nmolperday log_Total_2MeOE_0_nmolperday
log_Total_4MeOE_0_nmolperday
= age_strata | BMI_strata | trt / ss3;
run;
quit;

/*Per Will Thomas, these variables should be run with a simplified model. Keeping the trt interaction means we should present the comparisons in each of 6 age-bmi strata separately*/

/* PURPOSE: Conduct baseline comparisons (nmol/day) using a reduced model for all variables*/
Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model log_E1_0_nmolperday log_E2_0_nmolperday log_E3_0_nmolperday
log_16_OH_E1_0_nmolperday
log_OH_2_E1_0_nmolperday log_OH_2_E2_0_nmolperday log_OH_4_E1_0_nmolperday
log_OH_4_E2_0_nmolperday
log_MeO_2_E1_0_nmolperday log_MeO_2_E2_0_nmolperday log_MeO_4_E1_0_nmolperday
log_MeO_4_E2_0_nmolperday
log_R_2to16E1_0_nmolperday log_R_2to4E1_0_nmolperday
log_R_tot_2to4MeOE_0_nmolperday log_R_tot_2to4OH_E0_nmolperday
log_Total_2OHE_0_nmolperday log_Total_4OHE_0_nmolperday log_Total_2MeOE_0_nmolperday
log_Total_4MeOE_0_nmolperday
log MeO_2 E1_0 nmol per day log MeO_2 E2_0 nmol per day log MeO_4 E1_0 nmol per day  
log MeO_4 E2_0 nmol per day  
log R_2to16E1_0 nmol per day log R_2to4E1_0 nmol per day  
log R_tot_2to4OHE_0 nmol per day log R_tot_2to4MeOE_0 nmol per day  
log Total_2OHE_0 nmol per day log Total_4OHE_0 nmol per day log Total_2MeOE_0 nmol per day  
log Total_4MeOE_0 nmol per day  
= trt lean mass_0 age_strata | BMI_strata/ss3;  
lsmeans trt / CL pdiff;  
ODS output LSMeanCL=logCI_0;  
run;  
/*Significant difference at baseline in 2/16 p=0.043, 2OHE1 p=0.084*/  
quit;  
Data a;  
Set logCI_0;  
geometric_mean= exp(lsmean);  
left_CI= exp(LowerCL);  
right_CI= exp(UpperCL);  
drop lsmean LowerCL UpperCL;  
run;  
proc print data =a;  
run;  
/* PURPOSE: To conduct follow up comparisons (nmol/day) using a full model  
Also, adjustment for baseline 2OHE1 and 2/16 is necessary*/  
Proc GLM data=EM.EM_Merged_nmolperday_LowSTD;  
class age_strata bmi_strata trt;  
model log E1_1 nmol per day log E2_1 nmol per day log E3_1 nmol per day  
log 16 OH E1_1 nmol per day  
log OH_2 E1_1 nmol per day log OH_2 E2_1 nmol per day log OH_4 E1_1 nmol per day  
log OH_4 E2_1 nmol per day  
log MeO_2 E1_1 nmol per day log MeO_2 E2_1 nmol per day log MeO_4 E1_1 nmol per day  
log MeO_4 E2_1 nmol per day  
log R_2to16E1_1 nmol per day log R_2to4E1_1 nmol per day  
log R_tot_2to4OHE_1 nmol per day log R_tot_2to4MeOE_1 nmol per day  
log Total_2OHE_1 nmol per day log Total_4OHE_1 nmol per day log Total_2MeOE_1 nmol per day  
log Total_4MeOE_1 nmol per day  
= age_strata | BMI_strata | trt/ ss3;  
run;  
quit;  
/*Per Will Thomas, these variables should be run with a simplified model. Keeping the trt interaction  
means we should present the comparisons in each of 6 age-bmi strata separately*/  
/* PURPOSE: Conduct follow up comparisons (nmol/day) using a reduced model for all variables.  
Also, adjustment for baseline 2OHE1 and 2/16 is necessary*/  
Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;  
class age_strata bmi_strata trt;  
model log E1_1 nmol per day log E2_1 nmol per day log E3_1 nmol per day  
log 16 OH E1_1 nmol per day  
log OH_2 E1_1 nmol per day log OH_2 E2_1 nmol per day log OH_4 E1_1 nmol per day  
log OH_4 E2_1 nmol per day  
log MeO_2 E1_1 nmol per day log MeO_2 E2_1 nmol per day log MeO_4 E1_1 nmol per day  
log MeO_4 E2_1 nmol per day  
log R_2to16E1_1 nmol per day log R_2to4E1_1 nmol per day  
log R_tot_2to4OHE_1 nmol per day log R_tot_2to4MeOE_1 nmol per day  
log Total_2OHE_1 nmol per day log Total_4OHE_1 nmol per day log Total_2MeOE_1 nmol per day  
log Total_4MeOE_1 nmol per day  
= age_strata | BMI_strata | trt/ ss3;
\[ \text{log}_\text{MeO}_2 \text{E1}_1 \text{_nmolperday} \quad \text{log}_\text{MeO}_2 \text{E2}_1 \text{_nmolperday} \quad \text{log}_\text{MeO}_4 \text{E1}_1 \text{_nmolperday} \quad \text{log}_\text{MeO}_4 \text{E2}_1 \text{_nmolperday} \quad \text{log}_\text{R}_\text{2to4E1}_1 \text{_nmolperday} \quad \text{log}_\text{R}_\text{tot}_\text{2to4OHE}_1 \text{_nmolperday} \quad \text{log}_\text{R}_\text{tot}_\text{2to4MeOE}_1 \text{_nmolperday} \quad \text{log}_\text{Total}_\text{2OHE}_1 \text{_nmolperday} \quad \text{log}_\text{Total}_\text{4OHE}_1 \text{_nmolperday} \quad \text{log}_\text{Total}_\text{2MeOE}_1 \text{_nmolperday} \quad \text{log}_\text{Total}_\text{4MeOE}_1 \text{_nmolperday} \]

\[ = \text{trt} \quad \text{lean_mass}_0 \quad \text{age}_\text{strata} \mid \text{BMI}_\text{strata} / \text{ss}_3; \]

\text{lsmeans} \quad \text{trt} / \text{CL pdiff};

\text{ODS} \quad \text{output} \quad \text{LSMean}_\text{CL}=\text{logCI}_1;

\text{run};

\text{quit};

\text{Data} \quad \text{b};

\text{Set} \quad \text{logCI}_1;

\text{geometric}_\text{mean}= \exp(\text{lsmean});

\text{left}_\text{CI}= \exp(\text{LowerCL});

\text{right}_\text{CI}= \exp(\text{UpperCL});

\text{drop} \quad \text{lsmean} \quad \text{LowerCL} \quad \text{UpperCL};

\text{run};

\text{proc} \quad \text{print} \quad \text{data} = \text{b};

\text{run};

\text{Proc} \quad \text{GLM} \quad \text{Data} = \text{EM.EM}_\text{Merged}\_\text{nmolperday}\_\text{LowSTD};

\text{class} \quad \text{age}_\text{strata} \quad \text{bmi}_\text{strata} \quad \text{trt};

\text{model} \quad \text{log}_\text{OH}_2 \text{E1}_1 \text{_nmolperday} = \text{trt} \quad \text{log}_\text{OH}_2 \text{E1}_0 \text{_nmolperday} \quad \text{age}_\text{strata} \mid \text{BMI}_\text{strata} / \text{ss}_3;

\text{lsmeans} \quad \text{trt} / \text{CL pdiff};

\text{ODS} \quad \text{output} \quad \text{LSMean}_\text{CL}=\text{logCI}_2;

\text{run};

/*\text{Significant difference at baseline in 2/16 p=0.048}*/

\text{quit};

\text{Data} \quad \text{c};

\text{Set} \quad \text{logCI}_2;

\text{geometric}_\text{mean}= \exp(\text{lsmean});

\text{left}_\text{CI}= \exp(\text{LowerCL});

\text{right}_\text{CI}= \exp(\text{UpperCL});

\text{drop} \quad \text{lsmean} \quad \text{LowerCL} \quad \text{UpperCL};

\text{run};

\text{proc} \quad \text{print} \quad \text{data} = \text{c};

\text{run};

\text{Proc} \quad \text{GLM} \quad \text{Data} = \text{EM.EM}_\text{Merged}\_\text{nmolperday}\_\text{LowSTD};

\text{class} \quad \text{age}_\text{strata} \quad \text{bmi}_\text{strata} \quad \text{trt};

\text{model} \quad \text{log}_\text{R}_\text{2to16E1}_1 \text{_nmolperday} = \text{trt} \quad \text{log}_\text{R}_\text{2to16E1}_0 \text{_nmolperday} \quad \text{age}_\text{strata} \mid \text{BMI}_\text{strata} / \text{ss}_3;

\text{lsmeans} \quad \text{trt} / \text{CL pdiff};

\text{ODS} \quad \text{output} \quad \text{LSMean}_\text{CL}=\text{logCI}_3;

\text{run};

/*\text{Significant difference at baseline in 2/16 p=0.048}*/

\text{quit};

\text{Data} \quad \text{d};

\text{Set} \quad \text{logCI}_3;

\text{geometric}_\text{mean}= \exp(\text{lsmean});

\text{left}_\text{CI}= \exp(\text{LowerCL});
right CL= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data =d;
run;
/* PURPOSE: To conduct changes from baseline comparisons (nmol/day) using a full model.
Since there were significant differences at baseline in 2OHE1 and 2/16 ratio, include baseline term in the model*/

Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model
  D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday
  D__16_OH_E1_nmolperday
  D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday D_OH_4_E2_nmolperday
  D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_MeO_4_E1_nmolperday
  D_MeO_4_E2_nmolperday
  D_R_2to4E1_nmolperday
  D_R_tot_2to4OH2E_nmolperday D_R_tot_2to4MeOE_nmolperday
  D_Total_2OH2E_nmolperday D_Total_4OH2E_nmolperday
  D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday
= age_strata | bmi_strata | trt /ss3;
lsmeans trt / stderr pdiff;
run;
quit;

Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model
  D_OH_2_E1_nmolperday = log_OH_2_E1_0_nmolperday age_strata | bmi_strata | trt /ss3;
lsmeans trt / stderr pdiff CL;
run;
quit;

Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model
  D_R_2to16E1_nmolperday = log_R_2to16E1_0_nmolperday age_strata | bmi_strata | trt /ss3;
lsmeans trt / stderr pdiff;
run;
quit;
/* PURPOSE: To conduct changes from baseline comparisons (nmol/day/mol) using a reduced model.
Since there were significant differences at baseline in 2/16 ratio, include baseline term in the model*/

Proc GLM Data =EM.EM_Merged_nmolperday_LowSTD;
class age_strata bmi_strata trt;
model
  D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday D__16_OH_E1_nmolperday
  D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday D_OH_4_E2_nmolperday
  D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_MeO_4_E1_nmolperday
  D_MeO_4_E2_nmolperday
  D_R_2to4E1_nmolperday
  D_R_tot_2to4OH2E_nmolperday D_R_tot_2to4MeOE_nmolperday
  D_Total_2OH2E_nmolperday D_Total_4OH2E_nmolperday
  D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday

= trt lean_mass_0 age_strata | bmi_strata /ss3;
lsmeans trt / stderr pdiff CL;
run;
quit;

**Proc GLM Data** = EM.EM_Merged_nmolperday.LowSTD;
class age_strata bmi_strata trt;
model D_OH_2_E1_nmolperday = trt log_OH_2_E1_0_nmolperday age_strata | bmi_strata /ss3;
lsmeans trt / stderr pdiff CL;
run;
quit;

**Proc GLM Data** = EM.EM_Merged_nmolperday.LowSTD;
class age_strata bmi_strata trt;
model D_R_2to16E1_nmolperday = trt log_R_2to16E1_0_nmolperday age_strata | bmi_strata /ss3;
lsmeans trt / stderr pdiff CL;
run;
quit;

/*PURPOSE: Investigate associations between changes in EM and changes in body composition*/

**Data** EM.bodycomp_covariates;
set WISER.Bodycomp_319;
keep ID D_fat_mass D_percent_bodyfat D_lean_mass D_percent_leanmass;
run;

**Data** EM_changes;
set EM.EM_Merged_nmolperday.LowSTD;
keep ID D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday
   D__16_OH_E1_nmolperday
   D_OH_2_E1_nmolperday D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday
   D_OH_4_E2_nmolperday
   D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_MeO_4_E1_nmolperday
   D_MeO_4_E2_nmolperday
   D_R_2to16E1_nmolperday D_R_2to4E1_nmolperday
   D_R_tot_2to4OHE_nmolperday D_R_tot_2to4MeOE_nmolperday
   D_Total_2OHE_nmolperday D_Total_4OHE_nmolperday
   D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday;
run;

**Data** WISER.EM_Assoc_changes;
merge EM_changes EM.bodycomp_covariates;
run;

**Proc Corr Data** = wiser.em_assoc_changes;
var
   D_E1_nmolperday D_E2_nmolperday D_E3_nmolperday D_16_OH_E1_nmolperday
   D_OH_2_E1_nmolperday D_OH_2_E2_nmolperday D_OH_4_E1_nmolperday
   D_OH_4_E2_nmolperday
   D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_MeO_4_E1_nmolperday
   D_MeO_4_E2_nmolperday
   D_R_2to16E1_nmolperday D_R_2to4E1_nmolperday
   D_R_tot_2to4OHE_nmolperday D_R_tot_2to4MeOE_nmolperday
   D_Total_2OHE_nmolperday D_Total_4OHE_nmolperday
   D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday
D_Total_2OHE_nmolperday D_Total_4OHE_nmolperday
D_Total_2MeOE_nmolperday D_Total_4MeOE_nmolperday;
with D_fat_mass D_percent_bodyfat D_lean_mass D_percent_leanmass;
run;

**Proc GLM**
Data = EM.EM_Merged_nmolperday;
class trt age_strata bmi_strata;
model /* log_E1_0_nmolperday log_E2_0_nmolperday log_E3_0_nmolperday
log_16_OH_E1_0_nmolperday
log_OH_2_E1_0_nmolperday log_OH_4_E1_0_nmolperday log_MeO_2_E1_0_nmolperday
log_MeO_2_E2_0_nmolperday
log_R_2to4E1_0_nmolperday */ log_R_2to16E1_0_nmolperday
= trt age_strata | BMI_strata / ss3;
run;
lsmeans trt / stderr pdiff CL;
ODS output LSMeanCL=logCI_0;
run;
/*Significant difference at baseline in log(2/16OHE1) p=0.048. Changes from baseline in 2/16 will need to
be additionally adjusted for baseline 2/16*/
quit;

**Data** a;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CL= exp(LowerCL);
right_CL= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data = a;
run;

**Proc GLM**
Data = EM.EM_Merged_nmolperday;
class trt age_strata bmi_strata;
model D_R_2to16E1_nmolperday = log_R_2to16E1_0_nmolperday trt age_strata | bmi_strata / ss3;
lsmeans trt / stderr pdiff;
run;
/*Exercisers significantly increase their ratio (p=0.034) which was different from the controls (p=0.033)*/
quit;

**Proc Freq**
Data=EM.EM_Merged_ngpermgCr;
Tables bmi_class * trt / nopercent norow nocol;
run;

**Data** normalBMI_nmolperday;
Set EM.EM_Merged_nmolperday;
if bmi_class = '1_Nonmal';
run;

**Proc GLM**
Data = normalBMI_nmolperday;
class trt age_strata bmi_strata;
model log_E1_0_nmolperday /*log_E2_0_nmolperday log_E3_0_nmolperday
log_16_OH_E1_0_nmolperday*/
log_OH_2_E1_0_nmolperday /*log_OH_4_E1_0_nmolperday log_MeO_2_E1_0_nmolperday
log_MeO_2_E2_0_nmolperday
log_R_2to4E1_0_nmolperday */ log_R_2to16E1_0_nmolperday
= trt age_strata | bmi_strata/ ss3;
lsmeans trt / CL pdiff;
ODS output LSMeanCL=logCI_0;
run;
quit;

Data normal;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCI UpperCL;
run;
proc print data =normal;
run;

Proc GLM Data = normalBMI_nmolperday;
class trt age_strata bmi_strata;
model D_E1_nmolperday /*D_E2_nmolperday D_E3_nmolperday D_16_OH_E1_nmolperday
*/D_OH_2_E1_nmolperday /*D_OH_4_E1_nmolperday D_MeO_2_E1_nmolperday
D_MeO_2_E2_nmolperday
D_R_2to4E1_nmolperday */ D_R_2to16E1_nmolperday
= trt age_strata | bmi_strata /ss3;
lsmeans trt / stderr pdiff;
run;
quit;

Data overwtBMI_nmolperday;
Set EM.EM_Merged_nmolperday;
if bmi_class = '2_Overwt';
run;

Proc GLM Data = overwtBMI_nmolperday;
class trt age_strata bmi_strata;
model log_E1_0_nmolperday /*log_E2_0_nmolperday log_E3_0_nmolperday
log_16_OH_E1_0_nmolperday*/
log_OH_2_E1_0_nmolperday /*log_OH_4_E1_0_nmolperday log_MeO_2_E1_0_nmolperday
log_MeO_2_E2_0_nmolperday
log_R_2to4E1_0_nmolperday */ log_R_2to16E1_0_nmolperday
= trt age_strata | bmi_strata/ ss3;
lsmeans trt / CL pdiff;
ODS output LSMeanCL=logCI_0;
run;
quit;

Data overwt;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data =overwt;
run;

Proc GLM Data = overwtBMI_nmolperday;
class trt age_strata bmi_strata;
model D_E1_nmolperday /*D_E2_nmolperday D_E3_nmolperday D_16_OH_E1_nmolperday */D_OH_2_E1_nmolperday /*D_OH_4_E1_nmolperday D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_R_2to4E1_nmolperday*/ D_R_2to16E1_nmolperday = trt age_strata |bmi_strata /ss3;
lsmeans trt / stderr pdiff;
run;
quit;

Data obeseBMI_nmolperday;
Set EM.EM_Merged_nmolperday;
if bmi_class = '3_Obese';
run;

Proc GLM Data =obeseBMI_nmolperday;
class trt age_strata bmi_strata;
model log_E1_0_nmolperday /*log_E2_0_nmolperday log_E3_0_nmolperday log_16_OH_E1_0_nmolperday */log_OH_2_E1_0_nmolperday /*log_OH_4_E1_0_nmolperday log_MeO_2_E1_0_nmolperday log_MeO_2_E2_0_nmolperday log_R_2to4E1_0_nmolperday*/ log_R_2to16E1_0_nmolperday = trt age_strata | bmi_strata/ ss3;
lsmeans trt / CL pdiff;
ODS output LSMeanCL=logCI_0;
run;
quit;

Data obese;
Set logCI_0;
geometric_mean= exp(lsmean);
left_CI= exp(LowerCL);
right_CI= exp(UpperCL);
drop lsmean LowerCL UpperCL;
run;
proc print data =obese;
run;

Proc GLM Data = obeseBMI_nmolperday;
class trt age_strata bmi_strata;
model D_E1_nmolperday /*D_E2_nmolperday D_E3_nmolperday D_16_OH_E1_nmolperday */D_OH_2_E1_nmolperday /*D_OH_4_E1_nmolperday D_MeO_2_E1_nmolperday D_MeO_2_E2_nmolperday D_R_2to4E1_nmolperday*/ D_R_2to16E1_nmolperday

264
= trt age_strata | bmi_strata /SS3;
lsmeans trt / stderr pdiff;
run;
quit;

Data quartiles;
set EM.EM_Merged_nmolperday;
strata_2to16E1 = ROUND (log_R_2to16E1_0_nmolperday,0.1);
run;

Proc Freq Data=quartiles;
tables trt*strata_2to16E1/ nopercent norow nocol;
run;

proc univariate data=quartiles;
var strata_2to16E1;
run;

/* Table 2. Changes in body weight, anthropometry, fitness,PA*/
Data EM.Table2_ngpermgCr;
merge WISER.Demographics_319 WISER.Bodycomp_319 Wiser.Bodyweights_319
WISER.FoodRecords_312 WISER.Fitness_319 WISER.SRPA_319;
by ID;
if ID= '2601' then DELETE;
if ID= '2622' then DELETE;
keep ID trt
  age age_strata bmi_strata bmi_class
  weight_0 weight_1 D_weight PD_weight BMI_1 D_BMI PD_BMI
  fat_mass_0 fat_mass_1 D_fat_mass PD_fat_mass lean_mass_0 lean_mass_1 D_lean_mass
  PD_lean_mass
  kcal_0 kcal_1 D_kcal PD_kcal
  Mets_0  Mets_1 D_Mets PD_Mets
  total_mets_week_0 total_mets_week_1 D_SRPA D_percent_SRPA ;
run;

Proc GLM Data=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
model Mets_0 Mets_1 D_mets PD_mets = trt | bmi_strata | age_strata ss3;*/
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;

Proc GLM Data=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
model weight_0 weight_1 D_weight PD_weight = trt | bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;
PROC GLM DATA=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
*model bmi_0 bmi_1 D_bmi PD_bmi = trt | bmi_strata | age_strata;
model bmi_0 bmi_1 D_bmi PD_BMI = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;

PROC GLM DATA=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
*model fat_mass_0 fat_mass_1 D_fat_mass PD_fat_mass = trt|bmi_strata|age_strata;
model fat_mass_0 fat_mass_1 D_fat_mass PD_fat_mass = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;

PROC GLM DATA=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
*model lean_mass_0 lean_mass_1 D_lean_mass PD_lean_mass = trt|bmi_strata|age_strata;
model lean_mass_0 lean_mass_1 D_lean_mass PD_lean_mass = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;

PROC GLM DATA=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
*model kcal_0 kcal_1 D_kcal PD_kcal = trt|bmi_strata|age_strata;
model kcal_0 kcal_1 D_kcal PD_kcal = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
ods select lsmeans;
run;

/*Self-reported physical activity won’t be reported*/
PROC GLM DATA=EM.Table2_ngpermgCr;
class trt bmi_strata age_strata;
*model total_mets_week_0 total_mets_week_1 D_srpa D_percent_SRPA = trt bmi_strata | age_strata;
model total_mets_week_0 total_mets_week_1 D_srpa D_percent_SRPA = trt bmi_strata | age_strata;
lsmeans trt / stderr pdiff;
/* none of the trt*strata interactions were significant so will be removed from model*/
*ods select lsmeans;
run;