SERUM ESTRADIOL LEVELS AND MENTAL HEALTH-RELATED QUALITY OF LIFE IN CANADIAN POSTMENOPAUSAL WOMEN: A CROSS-SECTIONAL STUDY

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science in Clinical Epidemiology

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Abstract

**Background:** Serum estradiol levels decline after menopause and the effect on mental health-related quality of life (MHR-QOL) is unclear.

**Objective:** To determine if there is an association between endogenous serum estradiol levels and MHR-QOL in healthy postmenopausal women.

**Methods:** This cross-sectional study used baseline Canadian data from the Mammary Prevention.3 trial. Serum estradiol was measured with liquid chromatography-tandem mass spectrometry. Outcomes for MHR-QOL were the Medical Outcomes 36-Item Short Form Health Survey (SF-36) Mental Health Inventory-5 (MHI-5), Mental Component Summary (MCS), and the Menopause-Specific Quality of Life Questionnaire (MENQOL)-psychosocial domain.

**Results:** There were no statistically significant associations between estradiol levels and MHR-QOL in univariate analyses (n=455). Multivariable linear regression predicted statistically significant differences in MCS ($R^2=0.10, P=0.03$) and MENQOL-psychosocial domain ($R^2=0.10, P=0.04$), however estradiol was not a significant predictor.
**Conclusions:** This study did not find a statistically significant association between endogenous serum estradiol levels and MHR-QOL in healthy postmenopausal women.
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Preface

This research study was non-experimental. Study subjects were identified solely by their study identification number in the data that I received. The study protocol was approved by the Research Ethics Boards at the University of Toronto (Protocol Reference Number 24814) and the University Health Network (UHN REB Number 09-0647-CE).

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Conflicts of interest:

None.
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1.0 Chapter 1: Background

1.1 Introduction

The average female life expectancy in Canada is 82 years. Canadian women live more than one-third of their lives after the cessation of their ovarian function, in a state of low estrogen (Fang & Millar, 2009). A decline in estradiol, a form of endogenous estrogen, has been thought to affect mental health-related quality of life, particularly resulting in low mood (Blumel et. al., 2000; Young et. al., 2000; Conde et al., 2006; Lasiuk & Hegadoren, 2007). However, studies to date have conflicting and inconclusive results because of methodological issues (Erdincler et. al., 2004; Almeida et. al., 2005). Furthermore, Canadian normative data have demonstrated that mental health-related quality of life in women over the age of 45 increases with increasing age (Hopman et. al., 2000). This cross-sectional study aims to clarify if there is an association between endogenous serum estradiol levels and mental health-related quality of life in healthy Canadian postmenopausal women.

1.2 Estrogen Changes and Postmenopause

Estrogen levels fluctuate across a woman’s lifecycle (Deecher & Dorries, 2007). Three forms of estrogen are produced in women: estrone (E1), 17-beta-estradiol (estradiol, E2), and estriol (E3) (Behl, 2001). During reproductive years, estradiol is the predominant estrogen and is produced mainly by the dominant follicle, with levels reported to range from 20 to 400 pg/mL during the menstrual cycle. After menopause, the ovaries synthesize less estrogens and estradiol decreases to lower levels, <59 pg/mL (Kratz et. al., 2004; Lobo, 2007). While estrone becomes the more abundant estrogen, estradiol remains the predominant intracellular estrogen and is over ten times more potent than estrone.

Approximately 5% of estradiol is derived from the peripheral conversion of estrone to estradiol in the liver and other tissues, and this conversion becomes the main source of estradiol in postmenopausal women (Lobo, 2007). Also, a small amount of estradiol is produced by the aromatization of testosterone (Judd et. al., 1982). During premenopause,
Estradiol follows a diurnal variation with a peak in the morning and a trough in the later afternoon, while after menopause, estradiol levels are pulsatile but have less variability (Bao et. al., 2003; Murphy et. al., 2007; Hankinson et. al., 1995). Small amounts of circulating estradiol are free and can diffuse across the cell membrane to exert a biological effect, while the rest is bound to sex hormone-binding globulin (SHBG) and albumin. Estradiol is bound to SHBG with high affinity and loosely bound to albumin, while estrone is loosely bound to SHBG and has a greater affinity for albumin. Circulating SHBG is increased in hyperthyroidism and decreased in obesity, androgen excess, and hypothyroidism (Wu et. al., 2001; Lobo, 2007). Therefore, sex hormone-binding globulin levels impact the concentration of unbound estradiol, the compound that exerts a biological effect (Fortunati et. al., 2010).

Estrone (E1) is the second most abundant human estrogen with levels ranging from 1.5-170 pg/mL during the menstrual cycle. It is metabolized from estradiol as well as from the aromatization of androstenedione in adipose tissue and this conversion rate increases with age and weight. Estriol (E3) is produced by the placenta during pregnancy and from hydroxylation of estrone (Behl, 2001; Lobo, 2007).

### 1.3 Serum Estradiol Analysis Techniques

Measurement of serum estradiol is challenging in postmenopausal women because of the low physiological concentration of the hormone. Several methods have been used for the analysis of serum estradiol, including direct and indirect immunoassays and gas and liquid chromatography with tandem mass spectrometry. Important characteristics that distinguish the usefulness of these measurement techniques are the sensitivity, reproducibility, and accuracy of the methods, the amount of serum that is required to run the assay since there is often only a limited amount of serum available per person for analysis, and the cost of the analysis (Rinaldi et. al., 2001).

Direct immunoassays usually require only a small amount of serum sample, are fast, relatively inexpensive, can be automated, and do not require an extraction step (Rinaldi
et. al., 2001). However, they have been shown to be less sensitive in detecting low serum estradiol levels (Hogervorst et. al., 2003; Lee et. al., 2006). These direct immunoassays were initially developed to measure much higher hormone levels, such as in pregnancy. Indirect immunoassays have an additional purification step of chromatography separation that helps reduce particle interference to improve accuracy, however, this requires larger amounts of sample and is more labor intensive.

The “gold standard” methods for the measurement of serum estradiol levels have used mass spectrometry. Gas chromatography-tandem mass spectrometry (GC-MS/MS) improves on many of the limitations of the immunoassays, however, analysis times can be longer than 30 minutes for each sample. Therefore, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been shown to be a superior method in terms of sensitivity and efficiency (Nelson et. al., 2004; Kushnir et. al., 2008).

### 1.4 Mental Health-Related Quality of Life Measures and Correlation with Depression Scales

According to the World Health Organization, quality of life is defined as ‘an individual’s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns’ (WHOQOL, 1993). Health-related quality of life is a concept that specifically includes aspects of quality of life that relate to a person’s health and is a patient’s self-reported perception of their physical functioning, social functioning, role functioning, mental health, and general health (Wilson & Cleary, 1995). Mental health-related quality of life is one domain of health-related quality of life and is made up of several factors including depression (Ware & Sherbourne, 1992). Two health-related quality of life measures with mental health-related quality of life components that have been validated in Canadian postmenopausal women are the Medical Outcomes 36-Item Short Form Health Survey (SF-36) and the Menopause Specific Quality of Life Questionnaire (MENQOL) (Ware & Sherbourne, 1992; Hilditch et. al., 1996).
The SF-36 is a general health measure designed to evaluate physical and mental health as perceived by the patient. The SF-36 contains eight health dimensions that ask how much of the time over the past four weeks the person has experienced various symptoms (i.e., physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional health, and mental health) (Ware et. al., 1994). These domains can be scored individually or aggregated to produce physical and mental health summary scores, the Physical and Mental Component Summaries (PCS, MCS). The mental health measures of the SF-36, the Five-Item Mental Health Inventory (MHI-5) and Mental Component Summary (MCS), were developed for both discriminative and evaluative purposes to measure the emotional wellbeing in the general population for research studies and national health surveys (Ware & Sherbourne, 1992). The scale is scored out of 100 and a difference of 5 points on the scale is considered clinically meaningful (Bjorner et. al., 2007; Hopman et. al., 2010).

The MCS of the SF-36 has been shown to be highly correlated with the Montgomery-Asberg Depression Rating Scale (AUC: 0.85, 95% CI: 0.79-0.89) (Silveira et. al., 2005). Furthermore, the MHI-5 of the SF-36 has also been shown to be both a valid measure of mental health and to be highly correlated with the Diagnostic Interview Schedule for the diagnosis of major depressive disorder (AUC: 0.89, SE: 0.025) and with the Montgomery-Asberg Depression Rating Scale (AUC: 0.88, 95% CI: 0.83-0.93) (Berwick et al., 1991; Rumpf el. al., 2001; Silveira et. al., 2005). Cutoff scores of 52 and 42 on the MHI-5 and MCS, respectively, have been shown to indicate an increased likelihood of major depressive disorder (Silveira et. al., 2005). Higher SF-36 scores in any of the domains (MHI-5, MCS, and PCS) indicate a better mental health-related quality of life (Ware et. al., 2000; Hopman et. al., 2000).

The Menopause-Specific Quality of Life Questionnaire was developed specifically to measure health-related quality of life in postmenopausal women (Hilditch et. al., 1996). The questionnaire consists of 29 items divided into four domains: vasomotor, psychosocial, physical and sexual, with summary scores. The questionnaire asks whether
the person was bothered by the item in the past month, rated from 0 (not at all bothered) to 6 (extremely bothered). The scores are then converted to a range from 1 to 8 and a minimum clinically important difference is 1.0 point on the scale (Zollner et. al., 2005; Lucas et. al., 2009). The MENQOL has been validated in postmenopausal women and the psychosocial domain has been correlated with the General Well-Being Schedule ($R^2=0.7$) and the Geriatric Depression Scale ($R^2=0.38$, CI: 1.8-4.4) (Hilditch et. al., 1996; Kulasingam et. al., 2008). Higher scores on the MENQOL-psychosocial domain indicate a poorer mental health-related quality of life (Hilditch et. al., 1996).

1.5 Considering a Link Between Estrogen and Mental Health-Related Quality of Life

Estrogen exerts its effects by binding estrogen receptors (ER alpha and ER beta) on the cell membrane causing a protein cascade as well as by diffusing into cells and affecting gene transcription by binding to intracellular estrogen receptors (Behl, 2001). Estrogen has been shown to cause biochemical changes in the brain. In animal studies, estrogen was shown to modulate serotonergic, dopaminergic, and noradrenergic function in the brain by promoting serotonin synthesis, upregulating serotonin receptors, increasing noradrenaline turnover, and inhibiting monoamine oxidase activity (Woolley & McEwan, 1993; Belfort et al., 1995; Halbreich & Kahn, 2001; Lasiuk & Hegadoren, 2007). Clinical studies have demonstrated sex differences in response to tryptophan depletion, identifying a possible role for estrogen in modulating the serotonergic system (Payne et. al., 2009). The administration of estrogen and progesterone in postmenopausal women has also been shown to increase the density of serotonin receptors in the brain (Moses et. al., 2000). Furthermore, a decrease in hormone replacement therapy prescriptions has been shown to be associated with a significant increase in serotonergic antidepressant prescriptions suggesting that antidepressants are being prescribed for psychological or physical symptoms previously controlled for by hormone therapy (McIntyre et. al., 2005). These animal and clinical findings suggest that estrogen may have pharmacodynamic properties that are similar to antidepressant medications.
Nevertheless, the impact of estradiol on mental health-related quality of life is controversial. Most previous studies that have examined this relationship have focused on the menopausal transition (Freeman, 2010; Daly et. al., 2003; Bromberger et. al., 2003; Schmidt, 2004; Cohen et. al., 2006; Gallicchio et. al., 2007). Some cross-sectional studies have shown that women with a lifetime history of major depressive disorder have significantly lower pre- and perimenopausal estradiol (E2) levels and lower plasma estrone (E1) levels compared to non-depressed women (Harlow et. al., 2003; Ballinger, 1990). However, studies around the perimenopausal transition have not consistently found significant associations between endogenous estrogen levels and mood and well-being (Appendix 1).

In postmenopausal women, a systematic literature search revealed few studies that have examined differences in endogenous estrogen levels and mood and health-related quality of life (Appendix 2). Methodological problems in these studies include small sample sizes lacking sufficient power to detect an association between variables (Ballinger et. al., 1987; Ryan et. al., 2009), different sampling methods for hormone measurements (Ballinger, 1990; Woods et. al., 2007), poor sensitivity of the hormone assays used, which could often not detect estradiol levels below 20 pmol/L (5.4 pg/mL) (Barrett-Connor et. al., 1999; Ryan et. al., 2009), and a variety of outcome measures that are difficult to compare (Saletu et. al., 1996; Erdincler et. al., 2004). These factors may have contributed to conflicting results and may have obscured associations. In a larger cross-sectional study of Australian volunteers (n=265), age 70 years and older, postmenopausal women with lower endogenous estradiol levels were more likely to have depressive symptoms (Almeida et. al., 2005). However, only the results of unadjusted analyses were reported, therefore, possible confounding variables contributing to this finding could not be ruled out.
1.6 Covariates that may Influence Estradiol and Mental Health-Related Quality of Life

There are several measures that are considered confounding variables in the relationship between serum estradiol and mental health-related quality of life. These variables such as age, body mass index (BMI), smoking, the presence of a psychiatric comorbidity, and other hormones, may have an association with the study exposure, serum estradiol, and may also affect the outcome measure, mental health-related quality of life (Fletcher & Fletcher, 2005).

Increasing age has been shown to be associated with lower estrogen levels and better health-related quality of life in postmenopausal women (Deecher & Dorries, 2007; Conde et. al., 2006; Hopman et. al., 2000). Higher BMI is associated with elevated estrogen levels and lower health-related quality of life (Behl, 2001; Vasiljevic et. al., 2008). Smoking has been related to higher estrogen levels and lower health-related quality of life (Barret-Connor & Khaw, 1987; Zumoff et. al., 1990). Therefore, these variables can bias the relationship between serum estradiol and health-related quality of life.

The presence of a psychiatric comorbidity can be associated with lower health-related quality of life and can also be associated with reduced estradiol levels due to the effect of certain psychotropic medication on prolactin and estrogen suppression (Naidoo et. al, 2003). While low estrogen levels can result in hot flashes, which in turn can reduce health-related quality of life, the presence of hot flashes is an intermediary variable and is therefore, not included as a covariate (Deecher & Dories, 2007). Finally, cortisol is associated with serum estradiol levels and can affect health-related quality of life. Elevated cortisol levels caused by acute stress can reduce mood and memory retrieval (Dijkstra et. al., 2009). Cortisol can also decrease estradiol production by suppressing gonadotropin releasing hormone (GnRH) (Ballinger et. al., 1990; Mahesh & Brann, 1992).
1.7 Effects of other Sex Hormones on Mental Health-Related Quality of Life

In addition to estradiol, several other sex hormones, including estrone, testosterone, and dihydroepiandrosterone sulphate (DHEAS), have recently come under scrutiny for possibly playing a role in affecting mental health-related quality of life. Estrone is the most abundant estrogen after menopause. While it is physiologically less active than estradiol, it may have mood enhancing effects (Almeida et. al., 2005). The few studies that have previously investigated the effects of androgens (testosterone, DHEAS) on mood and health-related quality of life have produced inconsistent results (Cawood & Bancroft, 1996; Barrett-Connor et. al., 1999; Woods et. al., 2007; Almeida, 1999; Bromberger et. al., 2010). Therefore, these sex hormones require further exploration regarding the role they play in affecting mental-health related quality of life after menopause.

2.0 Chapter 2: Objectives and Hypotheses

The primary objective of this study is to determine if there is an association between serum estradiol levels and mental health-related quality of life as measured by the Medical Outcomes Study Short-Form Health Survey (SF-36) Five-Item Mental Health Inventory (MHI-5) and Mental Component Summary (MCS) scores.

Primary Hypothesis:
Serum estradiol levels will be significantly associated with mental health-related quality of life as measured by the MHI-5 and MCS, such that higher levels of serum estradiol will be associated with better mental health-related quality of life scores.

The secondary objective of this study is to determine if there is an association between serum estradiol levels and the Menopause-Specific Quality of Life Questionnaire (MENQOL)-psychosocial domain.
Secondary Hypothesis:
There will be an association between serum estradiol and the MENQOL-psychosocial domain, such that increased levels of serum estradiol will be associated with better mental health-related quality of life scores.

A Priori Exploratory Analyses: Associations between serum estradiol levels and the Physical Component Summary (PCS) of the SF-36 as well as relationships between serum estrone, testosterone, DHEAS, and cortisol with the SF-36 and MENQOL-psychosocial domain outcomes will also be explored.

Additional Exploratory Analyses:
Associations between serum estradiol and the MHI-5 and MCS using cutoff scores that indicate an increased likelihood of major depressive disorder, will be assessed. Subgroup analyses with women who underwent a natural and surgical menopause as well as with women with a history of previous use of hormone therapy will also be conducted to assess if these factors affect the relationship between serum estradiol and the MHI-5, MCS, and MENQOL-psychosocial domain.

3.0 Chapter 3: Methods

3.1 Study Design
This is a cross-sectional study using baseline Canadian data from the National Cancer Institute of Canada, Clinical Trials Group (NCIC-CTG) Mammary Prevention.3 trial (MAP.3). The MAP.3 study is an international, phase III, randomized, double-blind, placebo-controlled trial of exemestane versus placebo in healthy postmenopausal women at increased risk of breast cancer (Goss et. al., 2011). Data for this cross-sectional study are from the baseline visit of a random subset of the Canadian participants from the MAP.3 trial prior to participants taking any study medications and are described below (section 3.2).
3.2 Role of Principal Investigator

Prior to conducting this cross-sectional study and receiving the baseline Canadian data from the MAP.3 trial, I researched the various methods for analyzing estradiol levels and determined the best method for our study. I performed a background literature review and drafted a study protocol outlining the specific research question, methods, and analysis plans. The study protocol was approved by the University of Toronto Department of Health Policy, Management and Evaluation Clinical Epidemiology Program as well as by NCIC-CTG, Kingston, Ontario. I also applied for and received ethics approval from the Research Ethics Boards at the University of Toronto and the University Health Network. In addition, I applied for a research grant from the University Health Network, Department of Psychiatry Research Grant Competition, to support the serum analyses of estradiol and the other hormones. I arranged for the Mayo Clinic in Rochester, Minnesota, to carry out the sensitive hormone analyses outlined in the study and also coordinated the shipping of the serum samples from NCIC-CTG in Kingston, Ontario.

After I received the sociodemographic and health-related quality of life data from NCIC-CTG and the hormone quantification analyses from the Mayo Clinic, I started data cleaning, imputation, and statistical analyses with SAS 9.2 and presented the results to my committee for comments. I also applied for and received a CIHR-IGH travel award to support my presentation of the results at the 41st International Society of Psychoneuroendocrinology Annual Conference 2011, Berlin, Germany.

3.3 MAP.3 Trial

The MAP.3 trial is a randomized, double blind, placebo-controlled trial investigating the effects of exemestane, an aromatase inhibitor, compared to placebo, in a population of postmenopausal women at increased risk of breast cancer (Goss et. al., 2011). The primary outcome of the study was the incidence of invasive breast cancer. The study was conducted in Canada, the United States, Spain, and France and women were recruited between Feb. 11, 2004 and March 23, 2010. A total of 4560 women were included in the study, and 1285 women were from the Canadian arm. Women were eligible for the study
if they were postmenopausal (age >50 years and no spontaneous menses for >12 months, or ≤50 years and no spontaneous menses within 12 months and follicle-stimulating hormone level in the postmenopausal range or with prior bilateral oophorectomy).

Women also had at least one factor putting them at increased risk of breast cancer, such as being >60 years of age or having a Gail risk score >1.66%. Other risk factors can be found in the original paper (Goss et. al., 2011). Women were not considered eligible for the study if they were premenopausal, had previous breast cancer or other malignancies, were carriers of BRCA1 or BRCA2, or had uncontrolled thyroid, liver, or psychiatric disease.

At a baseline visit, prior to randomization, complete medical, reproductive, and family histories were obtained and women received a physical examination. Blood tests, bilateral mammography, and bone-mineral-density measurements were included from within the past year. Women were randomized to receive exemestane, exemestane plus celecoxib, or placebo, however, because of concern of increased cardiovascular disease with celecoxib, that arm was abandoned after approximately a year, and women randomized to that arm were included in the exemestane arm. Women were supposed to be followed for 5 years or until a breast or cardiovascular event, neoplastic disease, or toxicity occurred. It was an even-driven study and there were enough events after a median follow up of 3 years. Details of the baseline characteristics of the study subjects included in the MAP.3 trial are described in the original paper (Goss et. al., 2011). In summary, the median age of the participants was 62 years and most were white, with a median BMI of 28.

### 3.4 Study Participants

This study included healthy postmenopausal women, age ≥50 years and living in Canada who were recruited from the community through flyers, health fairs, community centres, physicians, media (radio, newspaper, television), and through the Ontario Breast Screening Registry for the MAP.3 trial.
After telephone screening to ensure inclusion and exclusion criteria for the MAP.3 trial, each subject received a baseline assessment where consent was obtained and health-related quality of life data were collected in the form of two self-report questionnaires, the SF-36 and the MENQOL (Goss et. al., 2011). Blood samples, height, weight, medical, lifestyle, and sociodemographic data were also collected at baseline for the MAP.3 cohort prior to participants taking any study medication. A sample of 480 women who met inclusion and exclusion criteria for this cross-sectional study (Appendix 3) was randomly selected from the Canadian MAP.3 study population using a random number generator in SAS 9.2.

### 3.5 Hormone Analyses

Frozen serum samples from these 480 women were stored in a freezer at -80° Celsius at NCIC-CTG in Canada in Kingston, Ontario. A total of 1mL of each sample was shipped to the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, for hormone analyses.

Total serum estradiol, estrone, testosterone, and cortisol were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404). Dihydroepiandrosterone sulfate (DHEAS) was measured by a competitive chemiluminescent immunoassay on the Immulite automated immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL 60015). Sex Hormone Binding Globulin (SHBG) was measured by a solid phase, two-site chemiluminescent assay on the Siemens Immulite 2000 automated immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL 60015). Intra-assay and inter-assay coefficients of variation (CV) ranged from 2.7-6.9% and 2.8-12%, respectively, and are provided below.

Estradiol: Intra-assay CVs are 3.1%, 5.0%, and 3.5% at 29, 109, and 325 pg/mL respectively. Inter-assay CVs are 8.6%, 9.0%, 6.6%, and 4.8% at 24, 61, 125 and 360 pg/mL, respectively.
Estrone: Intra-assay CVs are 3.4%, 5.1%, and 3.5% at 29, 109, and 332 pg/mL respectively. Inter-assay CVs are 6.9%, 5.2%, 6.3%, and 6.7% at 26, 58, 120, and 336 pg/mL, respectively.

Testosterone: Intra-assay CVs are 3.3%, 2.8%, 2.2%, and 2.0% at 16, 64, 184 and 927 ng/dL respectively. Inter-assay CVs are 5.1%, 3.8%, 3.7%, and 2.8% at 17, 65, 177, and 919 ng/dL, respectively.

Cortisol: Intra-assay CVs are 4.7%, 3.7% and 4.9% at 2.4, 10.7 and 22.0 mcg/dL respectively. Inter-assay CVs are 9.6%, 3.0% and 7.0% at 2.0, 10.0 and 20.5 mcg/dL, respectively.

Dihydroepiandrosterone Sulphate: Intra-assay CVs are 6.4% and 6.9% at 0.74 and 2.83 ug/mL. Inter-assay CVs are 12%, 10% and 10% at 0.49, 1.98, and 6.25 ug/mL, respectively.

Sex Hormone Binding Globulin: Intra-assay CVs are 2.7% and 3.1 % at 5.5, and 95.9 nmol/L, respectively. Inter-assay CVs are 4.0 % at 5.4 and 5.9 % at 74 nmol/L.

3.6 Statistical Considerations

3.6.1 Statistical Analyses

Primary analyses involved the SF-36 scoring manual to calculate and linearly transform the MHI-5 and MCS scores from a scale of 5 to 20 to a scale of 0 to 100 (Ware et. al., 2000). Descriptive statistics were used to determine means and standard deviations. Univariate analyses were conducted to assess for correlations between estradiol and the mental health-related quality of life measures. Spearman correlations were used when there was evidence of non-normality in the variables. Potential confounding variables including age, BMI, smoking status, number of cigarettes smoked, psychiatric comorbidity, and other sex hormones were identified a priori and included in multiple linear regression analyses. Effect modification was assessed for in univariate analyses by
including a term for the interaction between serum estradiol levels and BMI, and serum estradiol levels and ‘years since menopause’. Any significant interactions were included in the multivariable model. Model assumptions were checked by plotting residuals by predicted values for the model outcomes.

Secondary analyses assessed for a correlation between serum estradiol levels and MENQOL-psychosocial domain. Multivariable linear regression analyses were used to control for covariates. Exploratory analyses identified a priori were conducted to assess for the relationship between serum estradiol and the PCS outcome as well as for associations between estrone, testosterone, DHEAS, and cortisol with the SF-36 (MHI-5, MCS, and PCS) and the MENQOL-psychosocial domain. Additional exploratory analyses also included univariate and multivariable linear regression models that adjusted for covariates.

Correlations between variables confirmed to have a relationship in the literature were analyzed to assess the quality of the data. The level of statistical significance was set at alpha (two sided) =0.05. No corrections were made for multiple comparisons (Rothman, 1990). The statistical package SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for the analyses.

### 3.6.2 Sample Size

The predictor and outcome variables (serum estradiol and mental health-related quality of life, respectively) were treated as continuous variables, therefore, a correlation coefficient was used in the sample size calculation for this cross-sectional study. We were able to analyze serum samples from 480 women. In the planned linear regression analysis, serum estradiol was the primary predictor, and covariates included age, BMI, smoking status and amount smoked, psychiatric comorbidity, serum estrone, testosterone, DHEAS, SHBG, and cortisol. It was assumed that the $R^2$ between an outcome (eg. MHI-5 or MCS) and the covariates alone was 20%. With a sample size of 480 women, we estimate that the minimum increase in $R^2$ attributable to estradiol that could be detected with 90%
power was 2.6% (alpha = 0.05). Therefore, while a minimum clinically relevant explanation of variance in health-related quality of life attributed to estradiol is 5%, we have 90% power to detect an $R^2$ as low as 2.6% (Bjorner et. al., 2007, Hopman et. al., 2000).

3.7 Data Cleaning and Simple Imputation

3.7.1 Hormones

A total of 480 vials of serum, each containing 1mL sample, were shipped to the Mayo Clinic for hormone analyses. The only hormone value that required minor imputation was DHEAS. There were 27 subjects who had a DHEAS reading of <15 mcg/dL and these were imputed to a value of 15 mcg/dL for inclusion in the analyses. Hormone values that were considered outliers based on standard lab values and the sample distributions were excluded (Kratz et. al., 2004) (Appendix 4).

3.7.2 Health-Related Quality of Life Outcomes

*MHI-5*

The MHI-5 score involves the summation of five questions (9b+9c+9d+9f+9h) within the SF-36 with the lowest and highest possible scores, 5 and 25, respectively. The data were then transformed to a score with a scale of 0 to 100 using the formula below (Ware et. al., 2000).

\[
\text{MHI-5 transformed score} = \frac{(\text{actual raw score} - \text{lowest possible raw score})}{\text{possible raw score range}} \times 100
\]

There were three subjects who required simple imputation for the MHI-5 score calculation (Figure 1). Two subjects had an improbable score of 9 for one of the questions (the highest possible individual question score is 5) and one subject had a missing score for one of the questions. It was decided to impute the average raw score of
3 for each of these three questions with missing or improbable values, allowing for a calculation of an overall MHI-5 score for these subjects.

**Figure 1.** MHI-5 simple imputation

<table>
<thead>
<tr>
<th>Identifier</th>
<th>ALL_ANS</th>
<th>LOCATION</th>
<th>Q9B</th>
<th>Q9C</th>
<th>Q9D</th>
<th>Q9F</th>
<th>Q9H</th>
<th>totoscoremhi</th>
<th>transfmhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>63270</td>
<td>Y</td>
<td>CLINIC</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>29</td>
<td>120</td>
</tr>
<tr>
<td>45986</td>
<td>Y</td>
<td>CLINIC</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>.</td>
<td>2</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>60769</td>
<td>Y</td>
<td>CLINIC</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>23</td>
<td>90</td>
</tr>
</tbody>
</table>

**MCS and PCS**
There were three subjects that required simple imputation for the MCS and PCS scores. These first two subjects each had two questions with missing values. The median and mode value from the study sample was used for these items to allow for the calculation of the MCS and PCS scores for these subjects. The third subject had three questions answered with an improbable value, therefore the median and mode value of the sample for each question was used to impute for these three questions as well, allowing for the calculation of an MCS and PCS score.

**MENQOL-psychosocial domain**
Ten subjects without a numerical score and thirty-four subjects with a numerical score were involved in simple imputation to allow their inclusion in the analyses. The MENQOL consists of several questions and the subject must answer if the question pertains to them by answering yes (Y) or no (N). If they answer Y, then they are to give a numerical score to describe the extent that question applies to them. If they answer N, then they are not supposed to give a numerical score (so the numerical score should be missing). The imputations involved changing a Y to an N to match whether a numerical score or missing value was encoded. Furthermore, thirty-four subjects had a numerical score with an N which was converted to a Y. All of these simple imputations were logical, did not change the numerical scores of the subjects, and allowed the calculation of the MENQOL –psychosocial domain scores. An example of this simple imputation is
provided in Figure 2. For example, for question four on the MENQOL, subject 34809 did not answer if they were bothered or not with their personal life (yes=Y or no=N). However, they gave a score of 2 on the scale of how dissatisfied they are with their personal life. Therefore, a Y was imputed instead of a blank for the initial part of question four, allowing for the calculation of a MENQOL-psychosocial domain score.

**Figure 2.** MENQOL-psychosocial domain simple imputation

<table>
<thead>
<tr>
<th>Ident</th>
<th>S S S S S S S S</th>
<th>S</th>
<th>C C C C C C C C</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HO HO HO HO HO HO HO HO</td>
<td>AR AR AR AR AR AR AR AR AR</td>
<td>DE DE DE DE DE DE DE DE DE 1 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 4 5 5 6 6 7 7 8 8 9 9 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34809</td>
<td>2 Y 2 N . N . N . Y 1 Y 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72805</td>
<td>Y 2 Y 4 N . Y 3 N . 1 N .</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.0 Chapter 4: Results

4.1 Study Population

A total of 455 women were included in the study. This sample size was determined from the total Canadian MAP.3 sample (Figure 3).

Figure 3. Flow chart of final study sample

The mean age of the study population was 61.0 ± 5.2 years (min 51.0, max 82.7). Most of the women were married (66.8%), white (96.9%), and well educated with a community college degree or higher education (72.6%). Psychiatric comorbidity was present in 16.3% of the women and all were described as having a stable status. The average BMI was 28.5 ± 5.8 kg/m² (min 17.2, max 49.2). Regarding smoking status, only 6.8% of the women were currently smoking with the remainder either not smoking or of unknown status. Half of the women had smoked more than 100 cigarettes in their lifetime (50.1%).
Other sociodemographic data are listed below in Table 1 (Appendix 5, Age distribution of study sample).

Table 1. Sociodemographic, health, and lifestyle characteristics of the study population (n=455)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)/ Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61.0 (5.2) (min 51.0, max 82.7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6 (0.1) (min 1.4, max 2.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.6 (15.6) (min 43.7, max 134.2)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>28.5 (5.8) (min 17.2, max 49.2)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>57 (12.5%)</td>
</tr>
<tr>
<td>Married</td>
<td>304 (66.8%)</td>
</tr>
<tr>
<td>Separated</td>
<td>9 (2.0%)</td>
</tr>
<tr>
<td>Single</td>
<td>53 (11.7%)</td>
</tr>
<tr>
<td>Widowed</td>
<td>32 (7.0%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>441 (96.9%)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>5 (1.1%)</td>
</tr>
<tr>
<td>Asian</td>
<td>7 (1.5%)</td>
</tr>
<tr>
<td>American Indian/Alaska Native</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>13 (2.9%)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>431 (94.7%)</td>
</tr>
<tr>
<td>Unknown/Missing</td>
<td>11 (2.4%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>6 (1.3%)</td>
</tr>
<tr>
<td>Some high school</td>
<td>27 (5.9%)</td>
</tr>
<tr>
<td>High school diploma</td>
<td>92 (20.2%)</td>
</tr>
<tr>
<td>Tech/Community College/Cégep</td>
<td>122 (26.8%)</td>
</tr>
</tbody>
</table>
Some University Bachelor’s Degree at University University degree above Bach 39 (8.6%) 104 (22.9%) 65 (14.3%)

Postmenopausal Status
1. ≥50 years, no spontaneous menses ≥12 months 428 (94.1%)
2. Bilateral Oophorectomy 27 (5.9%)

Smoking History
< 100 cigarettes in lifetime 227 (49.9%)
≥100 cigarettes in lifetime 228 (50.1%)

Currently Smoking
Yes 31 (6.8%)
No 197 (43.3%)
Unknown 227 (49.9%)

Ever Used Hormone Therapy
Yes 245 (53.9%)
No 210 (46.2%)

Psychiatric Comorbidity
Yes 74 (16.3%)
No 381 (83.7%)

Location of Questionnaire Completion
Clinic 379 (83.73%)
Home 71 (15.6%)
Unknown 5 (1.1%)

Values are presented as mean ± SD or n (%)

4.2 Exposure Measures: Hormone Descriptive Statistics

The mean and standard deviation of each of the hormone values are listed in Table 2. Logarithmic and square root transformation of the values provided approximate normal
distributions (Appendix 6, Hormone distributions). Estradiol levels ranged from 1.4-35 pg/mL with a mean value of 7.7 pg/mL (Figure 4).

**Figure 4.** Original and log transformed distribution of serum estradiol

a. Serum estradiol distribution

![Original estradiol distribution](image)

b. Log serum estradiol distribution

![Log estradiol distribution](image)
### Table 2. Hormone descriptive statistics (n=455)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Mean (SD)</th>
<th>Min, Max</th>
<th>Transformation to approximate normal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>7.70 pg/mL (5.05)</td>
<td>1.4, 35</td>
<td>Log</td>
</tr>
<tr>
<td>Estrone</td>
<td>28.44 pg/mL (13.89)</td>
<td>5.6, 99</td>
<td>Log</td>
</tr>
<tr>
<td>Estradiol + Estrone</td>
<td>36.14 pg/mL (18.09)</td>
<td>8, 134</td>
<td>Log</td>
</tr>
<tr>
<td>DHEAS</td>
<td>77.26 mcg/dL (48.07)</td>
<td>15, 331</td>
<td>Log</td>
</tr>
<tr>
<td>Testosterone</td>
<td>16.99 ng/dL (10.34)</td>
<td>2, 72</td>
<td>Log</td>
</tr>
<tr>
<td>Cortisol</td>
<td>8.38 mcg/dL (3.07)</td>
<td>0.4, 20.1</td>
<td>Sqrt</td>
</tr>
<tr>
<td>SHBG</td>
<td>44.92 nmol/L (20.13)</td>
<td>11, 132</td>
<td>Log</td>
</tr>
</tbody>
</table>

### 4.3 Outcome Measures: Health-Related Quality of Life Descriptive Statistics

Descriptive statistics for mean values of the study outcome variables are outlined in Table 3. The MCS, PCS, and MENQOL-psychosocial domain scores do not demonstrate a clinically important difference from the age-matched standardized Canadian scores in postmenopausal women (Hopman et. al., 2000; Hilditch & Lewis, 1996). (Appendix 7, Health-related quality of life distributions).
Table 3. Health-related quality of life descriptive statistics (n=455)

<table>
<thead>
<tr>
<th>Quality of Life Measures</th>
<th>Mean (SD)</th>
<th>Min, Max</th>
<th>Transformation with log, square root, inverse did not achieve normal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHI-5</td>
<td>71.7 (9.3)</td>
<td>35, 95</td>
<td>Mildly skewed left</td>
</tr>
<tr>
<td>MCS</td>
<td>50.6 (9.3)</td>
<td>6.5, 72.7</td>
<td>Mildly skewed left</td>
</tr>
<tr>
<td>PCS</td>
<td>50.3 (8.8)</td>
<td>17.0, 67.2</td>
<td>Mildly skewed left</td>
</tr>
<tr>
<td>MENQOL-psychosocial domain</td>
<td>2.2 (1.1)</td>
<td>1.0, 7.6</td>
<td>Skewed right</td>
</tr>
</tbody>
</table>

4.4 Univariate Analyses: Correlations between Exposure and Outcome Measures

Each hormone was correlated with the health-related quality of life outcome measures (MHI-5, MCS, PCS, MENQOL-psychosocial domain) in univariate analyses. As the outcomes measures did not follow an approximate normal distribution, spearman correlation coefficients were calculated. There were no statistically significant correlations between any of the hormones and the MHI-5, MCS, or MENQOL-psychosocial domain outcomes in the univariate analyses (Table 4, Table 5). Estradiol, estrone, and the combination of estradiol and estrone were each statistically significantly negatively correlated with the PCS such that a lower hormone level correlated with a higher PCS score (Table 4).
Table 4. Univariate analyses of SF-36 health-related quality of life outcomes with hormone levels using Spearman correlation coefficients (r)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>MHI-5 (CI)</th>
<th>P value</th>
<th>MCS (CI)</th>
<th>P value</th>
<th>PCS (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (log)</td>
<td>-0.03 (-0.12, 0.06)</td>
<td>0.52</td>
<td>0.04 (-0.05, 0.14)</td>
<td>0.33</td>
<td>-0.12 (-0.21, -0.03)</td>
<td>0.01</td>
</tr>
<tr>
<td>Estrone (log)</td>
<td>0.05 (-0.05, 0.14)</td>
<td>0.34</td>
<td>0.09 (-0.003, 0.18)</td>
<td>0.06</td>
<td>-0.10 (-0.19, -0.01)</td>
<td>0.03</td>
</tr>
<tr>
<td>Estradiol + Estrone (log)</td>
<td>0.03 (-0.06, 0.12)</td>
<td>0.48</td>
<td>0.09 (-0.01, 0.18)</td>
<td>0.06</td>
<td>-0.11 (-0.20, -0.02)</td>
<td>0.02</td>
</tr>
<tr>
<td>DHEAS (log)</td>
<td>-0.01 (-0.10, 0.08)</td>
<td>0.81</td>
<td>0.02 (-0.07, 0.12)</td>
<td>0.62</td>
<td>0.08 (-0.01, 0.18)</td>
<td>0.07</td>
</tr>
<tr>
<td>Testosterone (log)</td>
<td>0.02 (-0.07, 0.11)</td>
<td>0.64</td>
<td>0.05 (-0.04, 0.14)</td>
<td>0.29</td>
<td>-0.04 (-0.13, 0.06)</td>
<td>0.45</td>
</tr>
<tr>
<td>Cortisol (sqrt)</td>
<td>-0.02 (-0.11, 0.08)</td>
<td>0.75</td>
<td>-0.03 (-0.12, 0.07)</td>
<td>0.57</td>
<td>0.11 (0.02, 0.20)</td>
<td>0.02</td>
</tr>
<tr>
<td>SHBG (log)</td>
<td>0.06 (-0.03, 0.15)</td>
<td>0.18</td>
<td>0.09 (-0.004, 0.18)</td>
<td>0.06</td>
<td>0.15 (0.06, 0.24)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
### Table 5. Univariate analysis of MENQOL-psychosocial domain outcomes with hormone levels using Spearman correlation coefficients (r)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>MENQOL-psychosocial domain (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (log)</td>
<td>0.02 (-0.07, 0.11)</td>
<td>0.68</td>
</tr>
<tr>
<td>Estrone (log)</td>
<td>-0.03 (-0.13, 0.06)</td>
<td>0.47</td>
</tr>
<tr>
<td>Estradiol + Estrone (log)</td>
<td>-0.03 (-0.12, 0.07)</td>
<td>0.58</td>
</tr>
<tr>
<td>DHEAS (log)</td>
<td>0.04 (-0.05, 0.13)</td>
<td>0.42</td>
</tr>
<tr>
<td>Testosterone (log)</td>
<td>-0.05 (-0.14, 0.04)</td>
<td>0.28</td>
</tr>
<tr>
<td>Cortisol (sqrt)</td>
<td>-0.08 (-0.17, 0.01)</td>
<td>0.09</td>
</tr>
<tr>
<td>SHBG (log)</td>
<td>-0.13 (-0.22, -0.04)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

#### 4.5 Identifying Effect Modifiers

**BMI**

Body mass index was investigated as an effect modifier for the relationship between serum estradiol and mental health-related quality of life. Since adipose tissue is an important source of conversion of estrone to estradiol in postmenopausal women, BMI may be an effect modifier, such that having greater amounts of adipose tissue (BMI ≥30) may alter the association between serum estradiol and mental health-related quality of life. Two BMI groups were created, BMI ≥30 (n=163) and BMI <30 (n=292) to assess for possible effect modification. There was no effect modification by BMI group, therefore, having a high or low BMI did not change the relationship between serum
estriadol level and the health-related quality of life outcomes (MHI-5, P=0.5; MCS, P=0.4; PCS, P=0.8; MENQOL, P=0.8).

**Years Since Menopause**

Number of years since menopause was investigated as an effect modifier on the relationship between serum estradiol level and mental health-related quality of life. The influence of estradiol may be different in the first ten years postmenopause since during this time the body is still adjusting to the decline in estrogen. A total of 428 women went through natural menopause with no spontaneous menses for at least 12 months by the time they entered the MAP.3 trial. Of these 428 women, 320 provided the date of their last menstrual period allowing for the calculation of the number of years since menopause (mean: 12.4 years ± 8.3). The number of years since menopause was divided into two groups: ≥10 years postmenopause (n= 172) and <10 years postmenopause (n=148). There was no effect modification by ‘years since menopause’ on the relationship between serum estradiol level and health-related quality of life outcomes (MHI-5, P=0.5; MCS, P=0.9; PCS, P=0.1; MENQOL, P=0.4).

### 4.6 Multivariable Analyses

Each of the quality of life outcomes was included in a multivariable analysis that consisted of predictors that were determined a priori based on the literature and clinical plausibility of being possible confounding variables in the relationship between serum estradiol and mental health-related quality of life. The large sample size of 455 women permitted the analysis of continuous predictors to be subdivided into quartiles to determine if there were any trends in their association with the outcomes. The predictor variables included age, BMI, presence or absence of psychiatric comorbidity, current smoking status, amount of cigarettes smoked, and additional hormone values. The impact of all the covariates on the health-related quality of life outcomes, without the primary predictor, serum estradiol, were determined for each of the multivariable models (MHI-5: \( R^2=0.07 \), P=0.15; MCS: \( R^2=0.1 \), P=0.014; PCS: \( R^2=0.14 \), P<0.0001; MENQOL-psychosocial domain: \( R^2=0.09 \), P=0.03). With the inclusion of serum estradiol in the
models (Table 6), the $R^2$ increased on average by 0.005 across the models. The MCS, PCS and MENQOL-psychosocial domain models had an overall statistically significant association with the predictors (Table 6).

Table 6. Linear regression models of health-related quality of life outcomes with predictors including serum estradiol

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictors</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHI-5</td>
<td>age (quartile) + BMI (quartile) + psychiatric comorbidity + current smoking status + amount</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>MCS</td>
<td>comorbidity + current smoking status + amount + amount smoked + estradiol (quartile) + estrone (quartile) + cortisol (quartile) + testosterone (quartile) + DHEAS (quartile) + SHBG (quartile)</td>
<td>0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>PCS</td>
<td></td>
<td>0.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>MENQOL-psychosocial domain</td>
<td></td>
<td>0.10</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*MHI-5 Model*

The predictors in the MHI-5 model did not have a statistically significant overall effect in predicting MHI-5 outcomes (P=0.12) (Table 6). Serum estradiol was neither a statistically significant predictor for this model (P=0.2), nor did it demonstrate a significant trend with MHI-5 outcomes (P=0.08) (Figure 5).

*Figure 5.* Relationship between serum estradiol quartiles and MHI-5 in the multivariable model (trend, P=0.08)
Table 7. Parameter estimates for serum estradiol quartiles in the MHI-5 multivariable model

<table>
<thead>
<tr>
<th>Estradiol Quartile</th>
<th>Parameter Estimate</th>
<th>P value</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>3.5</td>
<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.6</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Quartile 3</td>
<td>2.2</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Quartile 4</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

**MCS Model**

For the MCS model, again serum estradiol was not a statistically significant predictor of the model (P=0.7), nor did it demonstrate a statistically significant trend with MCS (P=0.34) (Figure 6). Serum estrone, however, demonstrated a statistically significant trend with MCS outcome, such that greater estrone levels were associated with better MCS scores in the model (P=0.02) (Figure 7). Psychiatric comorbidity also had a statistically significant association with MCS outcome such that the presence of a psychiatric comorbidity predicted a 5.2 point reduction in the MCS outcome (p<0.0001).

**Figure 6.** Relationship between serum estradiol quartiles and MCS in the multivariable model (trend, P=0.34)
Table 8. Parameter estimates for serum estradiol quartiles in the MCS multivariable model

<table>
<thead>
<tr>
<th>Estradiol Quartile</th>
<th>Parameter Estimate</th>
<th>P value</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>1.9</td>
<td>0.28</td>
<td>0.66</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>0.3</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Quartile 3</td>
<td>0.6</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Quartile 4</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. Relationship between serum estrone quartiles and MCS in the multivariable model (trend, P=0.02)

PCS Model

For the PCS model, the predictors, BMI and psychiatric comorbidity, each had a statistically significant association with the PCS outcome (P=0.003, P=0.04). Each quartile of the BMI predictor was statistically significantly associated with the PCS outcome such that a greater BMI was associated with a poorer PCS score, with the fourth quartile as the reference (Quartile 1: P=0.001, Quartile 2: P=0.002, Quartile 3: P=0.004). The overall trend between BMI and PCS was also statistically significant (P=0.001) (Figure 8).
Figure 8. Relationship between BMI quartiles and PCS in the multivariable model
(trend, P=0.001)

![Figure 8](image)

The presence of a psychiatric comorbidity had a statistically significant effect in predicting PCS outcome, with no psychiatric comorbidity predicting an increase in 2.3 points in the PCS score (P=0.04), with the fourth quartile as the reference. Serum estradiol level was neither a statistically significant predictor in the PCS model (P=0.7), nor did it demonstrate a significant trend with PCS outcome (P=0.2) (Figure 9).

Figure 9. Relationship between serum estradiol quartiles and PCS in the multivariable model (trend, P=0.2)

![Figure 9](image)
Table 9. Parameter estimates for serum estradiol quartiles in the PCS multivariable model

<table>
<thead>
<tr>
<th>Estradiol Quartile</th>
<th>Parameter Estimate</th>
<th>P value</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>-1.9</td>
<td>0.23</td>
<td>0.65</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>-1.04</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Quartile 3</td>
<td>-0.45</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Quartile 4</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

**MENQOL-psychosocial domain Model**

The predictor, psychiatric comorbidity, had a statistically significant effect on MENQOL-psychosocial domain outcome. The presence of psychiatric comorbidity was associated with a 0.6 point increase in MENQOL-psychosocial domain score (p<0.0001). Serum estradiol level was neither a statistically significant predictor of the MENQOL-psychosocial domain model (P=0.5), nor did it demonstrate a significant trend with the MENQOL-psychosocial domain outcome (P=0.6) (Figure 10).

**Figure 10.** Relationship between serum estradiol quartiles and MENQOL-psychosocial domain in the multivariable model (trend, P=0.6)
Table 10. Parameter estimates for serum estradiol quartiles in the MENQOL-psychosocial domain multivariable model

<table>
<thead>
<tr>
<th>Estradiol Quartile</th>
<th>Parameter Estimate</th>
<th>P value</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>-0.18</td>
<td>0.41</td>
<td>0.51</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>-1.13</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Quartile 3</td>
<td>-0.26</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Quartile 4</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

4.7 Exploratory Analyses

4.7.1 MHI-5 and MCS Cutoff Scores

Scores on the MHI-5 or MCS that were $\leq 52$ or $\leq 42$, respectively, have been shown to indicate an increased likelihood of major depressive disorder being present (Silveira et al., 2005). Univariate analyses did not demonstrate a statistically significant difference in serum estradiol levels between subjects with mental health-related quality of life outcomes above and below these cutoff scores (MHI-5, $P=0.9$ ; MCS, $P=0.8$).

4.7.2 Subgroup Analyses

4.7.2.1 Women with Natural Menopause

There were 428 women who were identified as having a natural menopause (ie. nonsurgical menopause). Women with surgical menopause were excluded from this sub-analysis since they are thought to experience a more rapid decline in estrogen and may exhibit a different relationship between serum estradiol and mental health-related quality of life. A total of 320 of the women who experienced natural menopause provided the date of their last menstrual period allowing for the calculation of the number of years since menopause. The predictor, ‘years since menopause’, was included in the multivariable models for this subgroup analysis of 320 women who experienced natural menopause. The only model that statistically significantly predicted health-related quality
of life outcome was the PCS model (P=0.02) (Table 11). Body mass index was the only statistically significant predictor in the model for PCS (BMI, P=0.01).

### Table 11. Linear regression models for subgroup analyses of women who experienced natural menopause (n=320)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictors</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHI-5</td>
<td>years postmenopause (&lt;10 yrs, &gt;/=10yrs) + age (quartile) + BMI (quartile) + psychiatric comorbidity + current smoking status + amount smoked + estradiol (quartile) + estrone (quartile) + cortisol (quartile) + testosterone (quartile) + DHEAS (quartile) + SHBG (quartile)</td>
<td>0.11</td>
<td>0.29</td>
</tr>
<tr>
<td>MCS</td>
<td>(quartile) + BMI (quartile) + psychiatric comorbidity + current smoking status + amount smoked + estradiol (quartile) + estrone (quartile) + cortisol (quartile) + testosterone (quartile) + DHEAS (quartile) + SHBG (quartile)</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>PCS</td>
<td>(quartile) + BMI (quartile) + psychiatric comorbidity + current smoking status + amount smoked + estradiol (quartile) + estrone (quartile) + cortisol (quartile) + testosterone (quartile) + DHEAS (quartile) + SHBG (quartile)</td>
<td><strong>0.15</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>MENQOL-psychosocial domain</td>
<td>(quartile) + BMI (quartile) + psychiatric comorbidity + current smoking status + amount smoked + estradiol (quartile) + estrone (quartile) + cortisol (quartile) + testosterone (quartile) + DHEAS (quartile) + SHBG (quartile)</td>
<td>0.13</td>
<td>0.09</td>
</tr>
</tbody>
</table>

#### 4.7.2.2 Women Who Underwent Surgical Menopause

There were 27 women included in the study who had a bilateral oophorectomy as well as hysterectomy (surgical menopause) with a mean age of 58.7 ± 4.7 years. The mean estradiol and estrone levels were 7.4 ± 3.1 and 29.9 ± 12.3, respectively, which were similar to the mean estradiol and estrone levels in the total study population (estradiol t-test, P=0.8; estrone t-test, P=0.6). There was no statistically significant difference between the surgical menopause group and the group who went through natural menopause regarding the health-related quality of life outcomes (MHI-5, P=0.5; MCS, P=0.3; MENQOL-psychosocial domain, P=0.6; PCS, P=0.06).

#### 4.7.2.3 Women with a History of Hormone Therapy Use

There was a subgroup of 245 women who indicated a history of previous use of hormone therapy. Univariate analyses in this subgroup did not reveal statistically significant
associations between serum estradiol and mental health-related quality of life outcomes (MHI-5, P=0.9; MCS, P=0.7; MENQOL-psychosocial domain, P=0.8).

### 4.8 Testing for Collinearity and Model Assumptions

The variance inflation factor values (each <2.5) did not reveal any collinearity between predictor variables for any of the models. The variance inflation factor values for the predictors in the MCS multivariable model are provided in Table 12.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>P value</th>
<th>Variance Inflation Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.04</td>
<td>1.14</td>
</tr>
<tr>
<td>Psychiatric Comorbidity</td>
<td>0.0001</td>
<td>1.02</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>0.94</td>
<td>1.12</td>
</tr>
<tr>
<td>Cigarettes Smoked</td>
<td>0.48</td>
<td>1.11</td>
</tr>
<tr>
<td>BMI</td>
<td>0.65</td>
<td>1.22</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.11</td>
<td>1.38</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.03</td>
<td>1.38</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.81</td>
<td>1.21</td>
</tr>
<tr>
<td>DHEAS</td>
<td>0.29</td>
<td>1.15</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.83</td>
<td>1.14</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.70</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Scatter plots of the residuals were created to check the assumption of the linear regression models for each health-related quality of life outcome (Figure 11- Figure 14).
**Figure 11.** Scatter plot of residuals compared to predicted values for the MHI-5 multivariable model

**Figure 12.** Scatter plot of residuals compared to predicted values for the MCS multivariable model
Figure 13. Scatter plot of residuals compared to predicted values for the PCS multivariable model

Figure 14. Scatter plot of residuals compared to predicted values for the MENQOL-psychosocial domain multivariable model
4.9 Assessing for Confirmed Correlations

Assessing for simple correlations between predictors identified a strong and positive statistically significant correlation between serum estradiol and estrone and also between each serum estradiol and estrone and BMI. Testosterone and DHEAS also had a positive and statistically significant correlation (Table 13). Increasing age was associated with better mental health-related quality of life outcomes and lower PCS scores (Table 14).

**Table 13.** Expected correlations between exposure variables

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Pearson correlation coefficient (r)</th>
<th>Spearman correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estrone</td>
<td>Testosterone</td>
</tr>
<tr>
<td>Estradiol</td>
<td>r=0.71 (CI 0.66, 0.75) p&lt;0.0001</td>
<td>r=0.45 (CI 0.37, 0.52) p&lt;0.0001</td>
</tr>
<tr>
<td>Estrone</td>
<td>r=0.47 (CI 0.39, 0.53) p&lt;0.0001</td>
<td>r=0.26 (CI 0.12, 0.34) p&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>r=0.29 (CI 0.20, 0.37) p&lt;0.0001</td>
<td>r=0.20 (CI 0.10, 0.28) p&lt;0.0001</td>
</tr>
<tr>
<td>DHEAS</td>
<td>r=0.03 (CI -0.06, 0.13) p=0.50</td>
<td>r= -0.31 (CI -0.37, -0.20) p&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>r= 0.04 (CI -0.05, 0.13) p=0.45</td>
<td></td>
</tr>
</tbody>
</table>
Table 14. Expected correlations between age and health-related quality of life outcomes with Spearman correlation coefficients (r)

<table>
<thead>
<tr>
<th>Variable</th>
<th>MHI-5</th>
<th>MCS</th>
<th>MENQOL-psychosocial</th>
<th>PCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$r=0.09$</td>
<td>$r=0.10$</td>
<td>$r=-0.13$</td>
<td>$r=-0.03$</td>
</tr>
<tr>
<td></td>
<td>(CI -0.01, 0.065)</td>
<td>(CI 0.01, 0.19)</td>
<td>(CI -0.22, -0.38)</td>
<td>(CI -0.12, 0.06)</td>
</tr>
<tr>
<td></td>
<td>$P=0.07$</td>
<td>$P=0.03$</td>
<td>$P=0.006$</td>
<td>$P=0.54$</td>
</tr>
</tbody>
</table>

5.0 Chapter 5: Discussion, Limitations and Future Directions

5.1 Summary and Strengths

The association between serum estradiol and mental health-related quality of life after menopause has not previously been adequately explored due to the few number of studies in the area and their methodological limitations (Appendix 2). The purpose of this study is to clarify if there is an association between serum estradiol and mental health-related quality of life in postmenopausal women. Gaining a better understanding of whether estrogen mediates effects on mental health-related quality of life could help identify women who might be at increased risk for mental health difficulties due to lower serum estradiol levels, either naturally, surgically, or induced by medications such as by an aromatase inhibitor (Lonning & Geisler, 2010). Furthermore, if an association is found, it would justify further studies of estrogen modifying agents and their effect on mental health-related quality of life (Rudolph et. al., 2004; Rasgon et. al., 2007). One approach to developing newer therapeutic strategies for mood disorders has been to identify subpopulations with unique biological or clinical markers who are more likely to respond to certain treatments (Papakostas & Fava, 2008). Therefore, a better understanding of the relationship between serum estradiol and mental health-related quality of life could clarify whether estradiol is a mediator or correlate with mental health-related quality of life in the postmenopausal population. However, the results of this well powered cross-sectional study, with a large sample size, sensitive hormone analysis techniques, and controlling for important confounders, did not demonstrate a statistically significant
association between serum estradiol and mental health-related quality of life in healthy postmenopausal Canadian women.

The study sample was representative of a healthy population of study volunteers with health-related quality of life scores within the normal ranges of healthy subjects (Hopman et. al., 2000; Hilditch et. al., 1996). Most of the surveys were completed in the clinic in a quiet setting which reduced potential measurement bias (Table 1) (Miller et. al., 2005). The highly specific and sensitive hormone assay technique of LC-MS/MS was used for the serum samples of this postmenopausal population where estradiol concentrations were low (Wu et. al., 2001; Hogervorst et al., 2003). The hormone levels found in this study are generally comparable to laboratory reference values (Kratz et. al., 2004). Correlations between predictors that have been replicated in the literature were tested (Table 13) and also found in this study. Positive and statistically significant correlations were demonstrated between each, estradiol and estrone, testosterone and DHEAS, BMI and estradiol, and BMI and estrone (Behl, 2001; Lobo, 2007; Rozenberg et. al., 1990). Also, similar to the Canadian population normative data, increasing age was associated with better mental health-related quality of life scores across all outcomes (MHI-5, MCS, and MENQOL-psychosocial domain) and was negatively associated with PCS scores (Hopman et. al., 2000). These factors help with the face validity and generalizability of the study.

5.2 Discussion of Primary, Secondary and Exploratory Outcomes

Primary and Secondary Outcomes

There were no statistically significant associations between serum estradiol levels and the mental health-related quality of life measures, MHI-5, MCS, or MENQOL-psychosocial domain (Table 4, Table 5). The confidence intervals were narrow which rules out large effects (Table 4, Table 5). While the direction of the correlation between serum estradiol and the MHI-5 was in the opposite direction to what was expected (a lower serum
estradiol level was associated with a better MHI-5 score (r=-0.03, R²=0.001)), this very small correlation is neither clinically meaningful nor statistically significant (P=0.52).

Multivariable analysis for the primary and secondary outcomes included predictors that were identified a priori as potential confounding and clinically relevant variables that could influence the relationship between serum estradiol levels and mental health-related quality of life outcomes. The suspected effect modifiers, BMI and ‘years since menopause’, were not confirmed to have such an effect on the mental health-related quality of life outcomes. Therefore, they were not included in the models as effect modifiers (BMI: (MHI-5, P=0.5; MCS, P=0.4; PCS, P=0.8; MENQOL, P=0.8, ‘years since menopause’: MHI-5, P=0.5; MCS, P=0.9; PCS, P=0.1; MENQOL, P=0.4). The variance inflation factor values (<2.5) for each of the predictors did not reveal any collinearity between variables, therefore, allowing the predictors to remain in the models (Table 12). Finally, the scatter plots of the residuals of the model predictors revealed model assumptions that were reasonably well met (Figures 11- Figure 14).

In the multivariable analysis of the relationship between serum estradiol levels and MHI-5, neither serum estradiol levels nor the other model predictors significantly predicted MHI-5 outcome. The lack of a significant finding can be due to the limited spread of the MHI-5 distribution. The MHI-5 only consists of five questions and may not have captured enough variability in scores to detect an association. The MHI-5 distribution was peaked with 70% of scores between a value of 70 and 80 (kurtosis 1.97) (Figure 15).
In the multivariable analysis, the MCS and MENQOL-psychosocial domain outcomes each had 10% of their variability determined by the predictors and this was statistically significant (P=0.03, P=0.04, respectively) (Table 6). Serum estradiol was neither a statistically significant predictor for the mental health-related quality of life outcome models nor did it display a significant trend with these outcomes (Figures 5, 6, and 10).

However, serum estrone demonstrated a statistically significant and clinically meaningful overall trend in predicting MCS outcome such that increasing estrone levels were associated with a better MCS score (trend, P=0.02) (Figure 7). Serum estrone, while less physiologically active than estradiol, is the most abundant estrogen in women after menopause (Lasiuk & Hegadoren, 2007). Perhaps the amount of estrogen must reach a certain threshold before it exerts a measurable and meaningful effect on mental health-related quality of life. This may explain the lack of finding a significant association between serum estradiol levels and mental health-related quality of life in postmenopausal women in this study. Serum estradiol levels after menopause may be too low to exert a biological effect that can impact mental health-related quality of life. Therefore, higher serum estradiol levels that are closer to premenopausal levels, which
can be achieved with hormone (estrogen) therapy, may have an important effect on mental health-related quality of life and requires further study (Rudolph et. al., 2004).

In summary, the predictors identified a priori for the multivariable linear regression analyses explained 7%, 10%, 14%, and 9% of the variability in the MHI-5, MCS, PCS, and MENQOL-psychosocial domain, respectively, before including the primary predictor, serum estradiol. The inclusion of serum estradiol only increased the $R^2$ on average by 0.5%, which was neither statistically nor clinically significant.

**Exploratory Outcomes Identified A Priori**

Exploratory analyses assessing for an association between other hormones and the health-related quality of life measures identified some interesting correlations. Serum estradiol and estrone were each statistically significantly correlated with PCS outcome in the univariate analyses such that higher levels of serum estradiol and estrone were associated with a lower PCS score (Table 4). However, in the PCS multivariable model, neither serum estradiol nor serum estrone was a statistically significant predictor. This relationship could be explained by the presence of a potential confounding variable, BMI. In the univariate analyses, BMI was negatively correlated with PCS ($r=-0.3$, $P<0.0001$), such that a higher BMI was associated with lower physical health-related quality of life. BMI was also correlated with serum estradiol and estrone ($r=0.52$, $p<0.0001$; $r=0.42$, $p<0.0001$, respectively), such that a higher BMI was associated with higher levels of serum estradiol and estrone. Therefore, when controlling for BMI in the multivariable model, serum estradiol and serum estrone were no longer statistically significant predictors of PCS, while BMI continued to be a significant predictor of PCS outcome ($P=0.02$) (Figure 8).

A similar explanation can account for the finding of a statistically significant association between SHBG and the MENQOL-psychosocial domain in the univariate analysis. Higher levels of SHBG are associated with less available free estradiol and therefore, would be expected to correlate with poorer mental health-related quality of life (Wu et. al., 2001). However, greater SHBG levels in this study were associated with a better
mental health-related quality of life outcome (lower MENQOL-psychosocial domain score). Since SHBG was not found to be a significant predictor in the multivariable model for the MENQOL-psychosocial domain, this indicates that the univariate finding was likely attributed to confounding (Table 6).

Exploratory analysis revealed another significant predictor variable, psychiatric comorbidity. While this was a statistically significant predictor for poorer health-related quality of life for each of the MCS, PCS, and MENQOL-psychosocial domain outcomes (change in 5.2, 2.3, 0.6 points, respectively), only the effect on MCS is clinically meaningful, as it predicts a greater than 5 point change in MCS score (Bjorner et. al., 2007, Hopman et. al., 2000).

**Additional Exploratory Outcomes**

Additional analyses were conducted to explore possible interactions between serum estradiol and mental health-related quality of life that could have been obscured by the previous analyses. Subjects were divided into two groups according to MHI-5 and MCS cutoff scores indicating a higher likelihood of major depressive disorder. However, there were no statistically significant differences in serum estradiol levels between subjects above or below the cutoff scores for the MHI-5 or MCS. This additional approach to analyzing the data provides further support for the findings that there is no significant association between serum estradiol levels and mental health-related quality of life.

Analyses of the subgroup of women with natural menopause (n=320), which included the additional predictor ‘years since menopause’, no longer revealed statistically significant models for the MCS and MENQOL-psychosocial domain outcomes. Since the correlation coefficients were within a small difference ($R^2=0.03$) from the correlation coefficients for the larger study population, the non significant finding is likely because of the reduced sample size lacking sufficient power to detect an association.

Examination of the subgroup of women with surgical menopause did not demonstrate a significant difference in estrogen levels from the rest of the sample, nor did their serum estradiol levels show a statistically significant relationship with mental health-related
quality of life outcomes. Furthermore, previous use of hormone therapy, which identifies a subgroup of women with potentially increased sensitivity to low hormone levels, did not modify the relationship between serum estradiol and mental health-related quality of life.

5.3 Limitations and Future Directions

There are several potential limitations in this study which may have obscured finding an association between serum estradiol levels and mental health-related quality of life. First, the data used in this study were baseline data from the MAP.3 trial. While this was a unique opportunity to study a large community-based sample of well-characterized postmenopausal women, there are limitations to using existing data. One limitation is that the study subjects are women participating in a randomized controlled trial; as such, they may have a selection bias because they are healthier, have higher socioeconomic status, and less mental health related comorbidities.

The data selected for this study was predetermined, therefore, variables that would be helpful to this study, such as details of the psychiatric history and antidepressant use, as well as confounding variables, such as progesterone, were not collected (Mahesh et. al., 1992; Lobo et. al., 2007). With existing datasets, the quality of the data can be compromised (Hulley et. al., 2001); there can be missing or incorrect measurements. However, the data quality of existing variables was high with few outlying or missing values and only simple imputation was required for this study. Since the data are already collected, data dredging and fishing for significant findings can also be a concern. However, a thorough literature review and study protocol including identifying the research question, hypotheses, and analysis plan as well as ethics approval, were all completed prior to receiving the data for the study. Also, any analyses that were not identified a priori were described as post hoc analyses.

This observational study has a cross-sectional design which limits the ability to both establish a causal relationship and to control for the presence of unknown confounding
variables (Fletcher & Fletcher, 2005). Estrogen levels were measured at one time and were not consistently fasting or morning samples. Although serum estradiol levels have been shown to have less variability in postmenopausal women (Hankinson et. al., 1995), for women more recently postmenopausal, a single sample may not be representative of the individual hormone profile for each subject. This study consists of a community sample which increases the external validity of the study, however, the generalizability is limited by the fact that the study sample is comprised of healthy volunteers. Perhaps, in healthy women, low estrogen levels do not have a significant impact on mental health-related quality of life. Instead, this relationship may be evident only in women with a predisposed vulnerability to being sensitive to hormonal levels, such as those who have a history of premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD), or postpartum depression. However, there is conflicting evidence about such an association and the subgroup analysis in this study with previous hormone therapy users did not demonstrate a significant association (Payne et. al., 2007; Payne et. al., 2009, Harlow et. al. 1999; Schmidt et. al., 2004; Woods et. al., 2008). It is possible that women with this vulnerability were already taking hormone therapy (a study exclusion criteria), and were therefore not included in the present study (Appendix 3).

Another potential limitation of this study is that the MAP.3 cohort consists of women at increased risk for breast cancer which may impact the health-related quality of life measures. However, previous studies have not shown a significant difference in health-related quality of life outcomes in women at increased risk of breast cancer compared to normative data (Brandberg et. al., 2003). Furthermore, more than half of the participants who qualified for this study did so based on their age being over 60 years, which in itself increases breast cancer risk (Goss et. al., 2011). Finally, the mental health-related quality of life scales used in this study are proxy measures of major depressive disorder and are difficult to compare with other studies using different outcomes. They are also self report measures that rely on the subject to provide accurate responses. Although study subjects have a tendency to change their behaviour when being a target of special interest and attention in a study (Fletcher & Fletcher, 2005), it is less likely that they intentionally alter their response based on their serum estradiol levels.
Previous studies have postulated that it may not be the absolute estrogen level that is associated with mental health-related quality of life, but instead the amount of change in estrogen level within the individual subject over time that has an impact on mental health-related quality of life (Freeman et. al., 2007; Ryan et. al., 2009). Therefore, a longitudinal study focusing on the earlier postmenopausal years, when estradiol levels may still be declining, and measuring intrasubject changes in serum estradiol and the relationship with mental health-related quality of life or clinician rated interview scales, such as the Montgomery-Asberg Depression Ration Scale, would be beneficial.

5.4 Conclusions

The findings of this cross-sectional study do not support a statistically significant association between serum estradiol levels and mental health-related quality of life in healthy Canadian postmenopausal women. Although there are several limitations to the study design, we attempted to correct for several important methodological deficiencies in previous studies. As such, we provide the best data to date regarding whether there is a relationship between serum estradiol levels and mental health-related quality of life after menopause. Further research is required to assess if changes in serum estradiol levels over time affect mental health-related quality of life, and in subpopulations that may be more vulnerable to hormonal changes.
6.0 References


## 7.0 Appendices

**Appendix 1:** Perimenopausal women: Studies investigating the relationship between endogenous serum estradiol levels and mood and health-related quality of life

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Population</th>
<th>Sample</th>
<th>Measurement</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dennerstein et. al., 2007</td>
<td>Prospective cohort</td>
<td>Perimenopausal Caucasian Australian women, Population based sample of women age 45-55</td>
<td>N=204</td>
<td>E2, symptoms, self-rated health, mood, sexual function</td>
<td>Mood/well-being not associated with hormone levels. E2 associated with hot flushes, night sweats, sleep problems, vaginal dryness and self-rated health (R²=0.51)</td>
</tr>
<tr>
<td>Freeman et. al., 2006</td>
<td>Prospective cohort</td>
<td>Premenopausal population based sample from Penn Ovarian Aging Study, age 35-47 with no history of depression</td>
<td>N=231</td>
<td>E2, CES-D, PRIME-MD</td>
<td>Significant association between intrasubject variability in estradiol and elevated CES-D (&gt;16)</td>
</tr>
<tr>
<td>Freeman et. al., 2004</td>
<td>Prospective cohort</td>
<td>Premenopausal population based sample from Philadelphia County, age 35-47</td>
<td>N=436</td>
<td>E2, CES-D, PRIME-MD</td>
<td>Increased estradiol associated with increased CES-D scores, but not significant (P=0.05)</td>
</tr>
<tr>
<td>Santoro et. al., 2005</td>
<td>Prospective cohort</td>
<td>Premenopausal and early perimenopausal women, multiethnic community based sample age 42–52 across the United States</td>
<td>N=2961</td>
<td>Serum estradiol, CES-D, Ladder of Life scale</td>
<td>No significant association between depressive symptoms or quality of life and E2 in perimenopausal women</td>
</tr>
<tr>
<td>Harlow et. al., 2003</td>
<td>Prospective cohort</td>
<td>Pre- and perimenopausal women, community sample age 36 – 44 selected from Massachusetts metropolitan area</td>
<td>N=976</td>
<td>Serum estradiol, Structured Clinical Interview for DSM-IV, HAMD</td>
<td>Depressed women had increased risk of an earlier perimenopausal transition and lower E2 levels after adjusting for covariates</td>
</tr>
<tr>
<td>Schmidt et. al., 2002</td>
<td>Case-Control</td>
<td>Perimenopausal women, community sample of volunteers, age 40-55</td>
<td>N=42 (21)</td>
<td>Plasma estradiol, CES-D</td>
<td>No significant difference in E2 in depressed and non-depressed women (P=0.06)</td>
</tr>
<tr>
<td>Avis et. al., 2001</td>
<td>Prospective cohort</td>
<td>Pre-, peri-, and postmenopausal women, community sample of women age 34-53 living in Massachusetts</td>
<td>N=309</td>
<td>Estradiol level, CES-D, symptoms of: hot flushes or night sweats, and trouble sleeping</td>
<td>Level of E2 significantly associated with depressive symptomatology in unadjusted analyses, but not significant upon adjustment for trouble sleeping and hot flushes/night sweats</td>
</tr>
<tr>
<td>Cawood et. al., 1996</td>
<td>Prospective cohort</td>
<td>Peri- and postmenopausal women, community sample age 40-60</td>
<td>N=141</td>
<td>Plasma estradiol, estrone, Multiple Affect Adjective Check list, Frenken Sexual Experience Scales</td>
<td>No significant relationship between E1 or E2 and depression. Tiredness was significantly related to depression and positive affect. Tiredness was also positively correlated with hot flushes</td>
</tr>
<tr>
<td>Ballinger et. al., 1987</td>
<td>Cross-sectional study</td>
<td>Postmenopausal women from gynecological outpatient clinic and local factory</td>
<td>N=54</td>
<td>Serum estradiol, GHQ</td>
<td>No significant difference in E2 in between women with high and low GHQ scores</td>
</tr>
</tbody>
</table>

CES-D, Center for Epidemiologic Studies-Depression scale; E1, Estrone; E2, Estradiol; HAMD, Hamilton Depression Scale; PRIME-MD, Primary Care Evaluation of Mental Disorders; GHQ, General Health Questionnaire
## Appendix 2: Postmenopausal women: A comprehensive review of studies investigating the relationship between endogenous serum estradiol levels and mood and health-related quality of life

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Population</th>
<th>Sample</th>
<th>Measurement</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryan et. al., 2009</td>
<td>Post hoc analysis of a prospective cohort</td>
<td>Postmenopausal women recruited for the Melbourne Women’s Midlife Health Project, population based sample with mean age of 60 (SD 2.5 years)</td>
<td>N=138</td>
<td>Serum estradiol, CES-D</td>
<td>No significant associations found between depressive symptoms and absolute E2. Women with a decline in E2 had a significant increase in depressive symptoms</td>
</tr>
<tr>
<td>Woods et. al., 2007</td>
<td>Prospective cohort</td>
<td>Postmenopausal women recruited from the community for The Seattle Midlife Women’s Health Study (SMWHSS)</td>
<td>N=41</td>
<td>Urinary estrone glucuronide (correlated with serum estradiol), 3-Day Symptom Diary</td>
<td>No significant correlation between severity of depressed mood and urinary estrone glucuronide</td>
</tr>
<tr>
<td>Almeida et. al., 2005</td>
<td>Cross-sectional study</td>
<td>Postmenopausal women, mean age 74.6 years, recruited volunteers living independently in the metropolitan area of Perth, Western Australia</td>
<td>N=265</td>
<td>Serum estradiol, Beck Depression and Anxiety Inventories, SF-36 Health Survey</td>
<td>Women in the high E2 group had significantly lower BDI scores and were less likely to have depressive symptoms than women in the low E2 group</td>
</tr>
<tr>
<td>Erdinciler et. al., 2004</td>
<td>Cross-sectional study</td>
<td>Postmenopausal volunteers age 55-85, seen at a geriatric primary care clinic in Istanbul</td>
<td>N=74</td>
<td>Plasma estradiol, Geriatric Depression Scale, interview with DSM IV diagnostic criteria</td>
<td>Estradiol levels were not statistically significant between depressive and non-depressive groups</td>
</tr>
<tr>
<td>Barrett-Connor et. al., 1999</td>
<td>Cross-sectional study</td>
<td>Postmenopausal community dwelling women, age 50-90, from Rancho Bernardo cohort</td>
<td>N= 699</td>
<td>Plasma estradiol, estrone, Beck Depression Inventory</td>
<td>No statistically significant association or trend between plasma estrogen and BDI score</td>
</tr>
<tr>
<td>Saletu et. al., 1996</td>
<td>Cross-sectional study</td>
<td>Postmenopausal women age 45-60 referred to menopausal clinic</td>
<td>N=118</td>
<td>E2, HAMD, DSM-III-R, ICD-10 research criteria checklists</td>
<td>No significant correlation between E2 and depressive symptoms</td>
</tr>
<tr>
<td>Ballinger, 1990</td>
<td>Cross-sectional study</td>
<td>Postmenopausal women attending 2 menopause clinics (n=123) and shopping centres (n=164)</td>
<td>N=285</td>
<td>Urinary and plasma estradiol, estrone, Hamilton Depression Rating Scale</td>
<td>Significant difference between urinary (not serum) estrogen levels and Hamilton Depression Rating Scale Scores</td>
</tr>
<tr>
<td>Ballinger et. al., 1987</td>
<td>Cross-sectional study</td>
<td>Postmenopausal women from gynecological outpatient clinic and local factory, age ≥45 years</td>
<td>N=31</td>
<td>Serum estradiol, GHQ</td>
<td>Significant difference in E2 in between women with high and low GHQ scores (p&lt;0.001) in the early postmenopausal group (N=18), LMP 1-4 years. No significant difference in E2 and high or low GHQ in late postmenopausal group (N=13)</td>
</tr>
</tbody>
</table>

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; CES-D, Center for Epidemiologic Studies-Depression scale; E2, Estradiol; GHQ, General Health Questionnaire; HAMD, Hamilton Depression Scale
## Appendix 3: Inclusion and exclusion criteria for study population

| **Inclusion Criteria** | - postmenopausal as defined as: ≥50 years, no spontaneous menses for at least 12 months/bilateral oophorectomy  
| | - complete baseline questionnaires (both SF-36 and MENQOL)  
| | - 3 serum vials present at baseline  
| | - signed informed consent |
| **Exclusion Criteria** | - premenopausal status  
| | - uncontrolled hypothyroidism or hyperthyroidism  
| | - history of malignancy, DCIS  
| | - major medical or psychiatric illness judged by the investigator to preclude follow up or compliance with the larger study  
| | - treatment with hormone therapies within 3 months  
| | - treatment ongoing or within 3 months with hormonal therapies including but not limited to: lutenizing hormone releasing hormone analogs, Lupron, progestogens  
| | - treatment with any investigational drug within 30 days or 5 half lives of the study  
| | - treatment with systemic estrogenic, androgenic agents within 3 months prior to the study  
| | - use of endocrine therapy, hormones, selective estrogen receptor modulators, or any other medications that may have an effect on the study endpoint |
Appendix 4: Hormone samples: Imputation methods and removal of outliers

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Imputation</th>
<th>Outliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>None</td>
<td>Outliers &gt; 59 pg/mL</td>
</tr>
<tr>
<td>Estrone</td>
<td>None</td>
<td>Outliers &gt; 120 pg/mL</td>
</tr>
<tr>
<td>DHEAS</td>
<td>DHEAS &lt;15 mcg/dL for 27 subjects coded as 15 mcg/dL</td>
<td>None</td>
</tr>
<tr>
<td>Testosterone</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Cortisol</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>SHBG</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
**Appendix 5:** Age distribution of study population (n=455)
Appendix 6: Hormone distributions

a. Distribution of estrone

b. Distribution of log estrone
c. Distribution of DHEAS

![Graph of DHEAS distribution]

DHEAS (mcg/dL)

Percent

DHEAS

30 60 90 120 150 180 210 240 270 300 330

0 5 10 15 20 25 30

Percent

35

10.0

12.5

15.0

17.5

20.0

DHEAS (mcg/dL)

![Graph of log DHEAS distribution]

d. Distribution of log DHEAS

![Graph of log DHEAS distribution]
e. Distribution of testosterone

![Graph showing distribution of testosterone levels.]

f. Distribution of log testosterone

![Graph showing distribution of log testosterone levels.]
g. Distribution of cortisol

![Graph of cortisol distribution]

h. Distribution of square root cortisol

![Graph of square root cortisol distribution]
i. Distribution of SHBG

![Graph showing the distribution of SHBG.]

j. Distribution of log SHBG

![Graph showing the distribution of log SHBG.]

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Appendix 7: Health-related quality of life distributions

a. MHI-5

b. MCS
c. PCS

![Diagram for PCS]

**MENQOL-psychosocial domain**

![Diagram for MENQOL-psychosocial domain]