The Role of Estrogen in the Mood-Lowering Effects of Acute Tryptophan Depletion in Postmenopausal Women

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Abstract

Depression is a major mental health problem for women. Several lines of evidence suggest that fluctuating levels of estrogen associated with various reproductive events are related to changes in mood. It has been hypothesized that estrogen may exert its influence on mood via its effect on the serotonergic system – a system frequently implicated in the regulation of mood. The major goal of the following study was to elucidate further the role of estrogen in mood regulation. To this end, we examined the role of estrogen in the mood-lowering effect of Acute Tryptophan Depletion (ATD), a technique designed to cause a marked lowering of plasma and brain tryptophan, and therefore brain serotonin levels, so that the effects of decreased serotonin on mood can be studied directly. We hypothesized that 1) exogenous estrogen may protect against the mood-lowering effects of ATD in postmenopausal women and that 2) a history of affective disturbance, particularly reproduction-related affective disturbance, would be associated with greater vulnerability to ATD as predicted by the kindling model of depression. Fifty-eight postmenopausal women were randomly assigned to treatment with estrogen (0.625 mg Premarin) or placebo in the context of a prospective, double-blind, cross-over design. During the final two weeks of the 12-week treatment phase, all participants completed one ATD test session and one nutritionally balanced amino acid control session. We found that: 1) treatment with exogenous estrogen significantly improved mood and menopausal symptoms as compared to placebo treatment, 2) ATD was associated with a significant lowering of mood in both groups, 3) treatment with estrogen did not protect women from ATD effects unless they responded to 11 weeks of

treatment with exogenous estrogen with enhanced mood, and 4) a history of reproduction-related affective disturbance was associated with more dysphoric mood in response to ATD. In conclusion, these data provide further evidence for a protective role of estrogen in mood regulation and for the role of the reproductive hormones in the kindling process of affective disturbance in women.

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Résumé

La dépression est un problème de santé mentale important chez les femmes. Plusieurs éléments de preuve portent à croire que des niveaux d'estrogène fluctuants associés à divers événements reproductifs sont liés à des changements de l'humeur. L'estrogène pourrait exercer son influence sur l'humeur via son effet sur le système sérotonergique. L'objectif principal de la présente étude était d'élucider davantage le rôle de l'estrogène dans la régulation de l'humeur. Nous avons examiné le rôle de l'estrogène dans le contexte de l'effet de baisse de l'humeur de la Déplétion Aiguë de Tryptophane (DAT), une technique permettant de causer une baisse de sérotonine dans le cerveau. Nous avons formulé les hypothèses suivantes : 1) l'estrogène exogène pourrait protéger contre les effets de la DAT chez les femmes post-ménopausées ; 2) une histoire de troubles affectifs, plus particulièrement en lien avec la reproduction, serait associée à une plus grande vulnérabilité à la DAT, tel que prédit par le modèle de l'allumage ('kindling') de la dépression. Cinquante-huit femmes post-ménopausées ont été réparties de façon aléatoire à des groupes de traitement par estrogène (0.625 mg Premarin) ou placebo dans le cadre d'un plan prospectif, à double insu, avec alternance de traitements. Pendant les deux dernières semaines de la période de traitement de 12 semaines, toutes les participantes ont complété une séance expérimentale de DAT et une séance contrôle. Nous avons trouvé les résultats suivants : 1) le traitement à l'estrogène a amélioré l'humeur et les symptômes de ménopause de façon significative, 2) la DAT était associée à une baisse significative de l'humeur dans les deux groupes, 3) le traitement à l'estrogène n'a pas protégé les femmes des effets de la DAT à moins qu'elles n'aient

réagi à 11 semaines de traitement à l'estrogène par une amélioration de l'humeur, 4) une histoire de troubles affectifs liés à la reproduction était associée à une humeur dysphorique accrue en réaction à la DAT. En conclusion, ces résultats constituent une preuve supplémentaire du rôle protecteur de l'estrogène dans la régulation de l'humeur et du rôle des hormones reproductives dans le processus d'allumage ('kindling') des troubles affectifs chez les femmes.

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INTRODUCTION

Depression is a significant mental health problem for women. Epidemiological studies in Canada (Stephens, Dulberg, & Joubert, 1999) the U.S. (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Weissman, Leaf, Holzer, Myers, & Tischler, 1984) and cross culturally (Gater et al., 1998) consistently demonstrate a 2:1 female/male ratio in the prevalence of depressive disorders. Sex differences in the rates of depression are seen initially during adolescence (Buchanan, Eccles, & Becker, 1992; Weissman et al., 1987), a time of major changes in the neuroendocrine systems. The cyclical nature of the secretion of steroid hormones from puberty to menopause may contribute to women's vulnerability to mood disorders (Seeman, 1997). Steroid hormones produced by the ovaries include estrogens, progestins, and androgens. Several lines of evidence suggest that fluctuating levels of estrogen associated with various reproductive events are related to concurrent changes in mood.

Estrogen, Reproduction-Related Events, & Mood:

Menstrual Cycle:

During the follicular phase of the menstrual cycle, the ovaries secrete increasing amounts of estrogen and levels peak on the day of ovulation (approximately the 14^{th} day of the menstrual cycle). During the luteal phase, estrogen secretion decreases slightly and then there is another peak during the midluteal phase following which estrogen levels

decrease and reach a nadir at the onset of menstruation. Positive moods are most likely to occur during the late follicular (periovulatory) phase of the menstrual cycle when estradiol levels are increasing (Metcalf & Livesey, 1995). Conversely, negative moods often occur during the late luteal (premenstrual) phase of the cycle when estrogen levels are decreasing (Parry, 1992). It has been estimated that between 50-90% of women report at least mild mood or somatic changes premenstrually (Campbell, Peterkin, O'Grady, & Sanson-Fisher, 1997; Hylan, Sundell, & Judge, 1999; Johnson, McChesney, & Bean, 1988; Merikangas, Foeldenyi, & Angst, 1993; Singh, Berman, Simpson, & Annechild, 1998). This is true of North American samples and also occurs cross culturally (Monagle et al., 1993). In a recent national probability study in the United States, 41% of women self-diagnosed premenstrual syndrome, 58% reported premenstrual symptoms (PMS) and 48% reported feeling more emotional prior to menstruation (Singh et al., 1998). Given the frequent occurrence of these symptoms, they may be considered a common response to natural fluctuations in hormone levels and need not be regarded as a diagnostic category (Steiner, 1997).

A smaller percentage of women experience premenstrual symptoms that are of sufficient severity to interfere with social and occupational functioning. However, the lack of a common definition of premenstrual syndrome has made it difficult to assess its exact prevalence and may have led to overestimates of its occurrence. The inclusion of diagnostic criteria for Late Luteal Phase Dysphoric Disorder (LLPDD) in the appendix of the Diagnostic and Statistical Manual of Mental Disorders, third edition revised (DSM-III-R) (1987) and the more recent inclusion of research diagnostic criteria for

Premenstrual Dysphoric Disorder (PMDD) in the DSM-IV (1994) have provided guidelines for diagnosis and systematic research.

According to the DSM-IV, the symptoms of PMDD include the following: 1) feelings of sadness, hopelessness, or self-deprecation; 2) tension or anxiety; 3) marked mood lability with frequent tearfulness; 4) persistent irritability, anger and increased interpersonal conflicts; 5) decreased interest in usual activities which may be associated with withdrawal from social relationships; 6) difficulty concentrating; 7) fatigue, lethargy, or low energy; 8) marked changes in appetite, which may be associated with binge eating or craving certain foods; 9) hypersomnia or insomnia; 10) a subjective feeling of being overwhelmed or out of control; and 11) physical symptoms such as breast tenderness or swelling, headaches, or sensations of "bloating" or weight gain. These symptoms have been shown to be highly replicable across menstrual cycles (Bloch, Schmidt, & Rubinow, 1997) and the severity of these symptoms may be comparable to those of a major affective disorder (Rapkin, Reading, Woo, & Goldman, 1991).

In order to meet diagnostic criteria for PMDD, five or more of the above-listed symptoms (including at least one of the first four) must regularly occur during the last week of the luteal phase, must begin to remit within a few days of the onset of menstruation and must be completely absent in the week following menses in most menstrual cycles. The presence of the cyclical pattern of symptoms must be confirmed by at least 2 consecutive months of prospective daily symptom ratings. In general,

estimates of the incidence of PMDD range from 2-10% of women. For example, 4.6% of young college women (Rivera-Tovar & Frank, 1990) and 3.2% of women in a large stratified sample of nursing school graduates (Johnson et al., 1988) met criteria for the diagnosis of PMDD.

While the etiology of PMS and PMDD are still largely unknown, changes in production of the female sex hormones are clearly implicated. As early as 1931, Frank hypothesized that "premenstrual tension" was caused by an excess of estrogen (Frank, 1931). Since that time, numerous studies have reported contradictory findings. Some investigators have found a positive relationship between estradiol levels and symptoms of PMDD (Hammarback, Damber, & Backstrom, 1989; Redei & Freeman, 1995). Hammarback et al. (1989) found a positive relationship between combined high periovulatory and luteal phase estradiol and progesterone levels and symptom severity. That is, in those menstrual cycles when estradiol levels were higher in both phases of the menstrual cycle, PMDD symptoms were worse. Similarly, women categorized as having more severe PMDD have been shown to have higher levels of estrogen, which was most pronounced around the time of ovulation (Redei & Freeman, 1995). In contrast, lower estrogen levels have also been found in women with premenstrual symptoms when compared to healthy controls (Van Goozen, Wiegant, Endert, Helmond, & Van de Poll, 1997), and depressive affect has been shown to be negatively correlated with estradiol levels in depressed premenopausal women (Baischer, Koinig, Hartmann, Huber, & Langer, 1995). Still others have found no significant relationship between estrogen levels and PMDD (Dennerstein et al., 1993; Halbreich, Endicott, Goldstein, & Nee,

1986). For example, in a study of the urinary hormone profiles of women with and without PMDD, no differences in estrogen levels were demonstrated (Dennerstein et al., 1993).

Despite these inconsistencies, many authors maintain that gonadal hormones are likely contributors to the etiology of PMDD (Halbreich et al., 1986; Rubinow & Schmidt, 1995; Steinberg, 1991). This is largely due to the considerable evidence regarding the therapeutic efficacy of hormonal PMDD treatments (Rubinow & Schmidt, 1995). For example, treatment studies of PMDD have found that exogenous estrogen (administered either transdermally or via estradiol implants) causes significant decreases in negative mood symptomatology (Smith, Studd, Zamblera, & Holland, 1995; Watson, Studd, Savvas, & Baber, 1990). In addition, when surgical oophorectomy was performed in women with severe, treatment resistant PMDD and they were subsequently treated with continuous estrogen replacement, the cyclic pattern of mood change was eliminated and mood and overall quality of life were significantly improved (Casper & Hearn, 1990). Finally, GnRH agonists (which suppress ovulation by down-regulating secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) leading to decreased stimulation of the follicle and thus to decreased production of circulating estrogen and progesterone), also improve PMDD symptoms (Altshuler, Hendrick, & Parry, 1995). In a double-blind, placebo-controlled study of the effects of leuprolide acetate (Lupron), a GnRH agonist, on PMDD symptoms, almost all symptoms (including sadness, irritability and impaired functioning) were significantly improved during the equivalent of the late luteal phase (e.g. the fourth week of treatment) of the menstrual cycle compared both to

pre-treatment levels and to placebo controls. Estrogen levels were very low throughout the cycle in these Lupron-treated women and symptom incidence during the equivalent of the late luteal phase was not significantly different from that reported during the equivalent of the periovulatory phase (e.g. the second week of treatment) (Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998). The effect of GnRH agonists on PMDD may be limited to those women whose symptoms are restricted to the late luteal phase of the menstrual cycle, as opposed to those with ongoing dysphoria that is exacerbated premenstrually (Freeman, Sondheimer, & Rickels, 1997). Similarly, the effect of GnRH agonists on PMDD may be limited to those individuals with less severe PMDD (as defined by premenstrual Beck Depression Inventory scores of less than 19). The authors speculate that women with severe PMDD may have a more sensitive hypothalamic-pituitary-ovarian feedback system, which may be related to altered neurotransmitter responsiveness. This may have led to greater estradiol suppression, causing more toxic effects in women with severe PMDD (Brown, Ling, Andersen, Farmer, & Arheart, 1994).

The current consensus appears to be that PMDD is triggered in some women by the normal endocrine events of the ovarian cycle (rather than by a hormonal imbalance or abnormality) which causes a lowering of mood in those women who are sensitive via sensitization, kindling or conditioning (Rubinow & Schmidt, 1995).

Pregnancy & Postpartum:

The endocrine changes that occur during pregnancy and at parturition are unique with respect to the magnitude and the speed with which they occur (Campbell, 1992). Changes in gonadal steroid levels during pregnancy greatly exceed those that occur during the menstrual cycle and are larger than those which result from the suppression of these hormones during oral contraceptive therapy (Hamilton, Parry, & Blumenthal, 1988). Throughout pregnancy, estrogen and progesterone blood levels increase steadily, reaching the highest physiological levels ever experienced by healthy women. Upon delivery of the placenta, there is a rapid clearance of placental steroids. The concentrations of these hormones reach a nadir 2-7 days postpartum and remain depressed until resumption of ovarian follicular activity. Estrogen and progesterone concentrations are more than 200 times lower by the end of the first postnatal week compared to late pregnancy (Bonnar, Franklin, Nott, & McNeilly, 1975; Deakin, 1988).

Despite the massive increases in gonadal hormone concentrations, pregnancy is generally associated with a relatively low incidence of psychiatric disorders. In fact, the incidence of mental illness is substantially lower during pregnancy (7.1 per 10,000 woman years) compared with the incidence during non-childbearing periods (35.1 per 10,000 woman years) (Paffenbarger, 1982). Conversely, the most likely time for a woman to become depressed is during the postpartum period, when there is a threefold increase in risk (Cox, Murray, & Chapman, 1993; Paffenbarger, 1982). Postpartum illness may manifest itself in a variety of symptoms and syndromes, but the most

common syndrome of severe postpartum illness is depression. Severe postpartum affective syndromes are distinguished from maternity- or baby-blues dysphoria in terms of the severity and frequency of symptoms, the time course of the illness, and its epidemiology (Hamilton, 1989). Postpartum blues, characterized by mild depression, crying, irritability, anxiety, and fatigue, generally occurs on the third or fourth day after delivery. Symptoms are usually transitory, lasting from one day to up to two weeks. The reported incidence of postpartum dysphoria varies, but it appears to be between 50 and 80 percent (Buesching, Glasser, & Frate, 1986; Steiner, 1979). A more serious form of postpartum affective disturbance, postpartum depression (PPD), is characterized by symptoms of a major affective disorder. It may have a delayed onset (6 – 16 to weeks postpartum) and frequently resolves within 6-12 months following delivery (Cox et al., 1993; Georgiopoulos et al., 1999; Hamilton, 1989). PPD has been consistently demonstrated to occur in approximately 10-15% of women cross-culturally (Cox et al., 1993; Georgiopoulos et al., 1999; Hearn et al., 1998). In a population based screening for postpartum depression in the United States using the Edinburgh Postnatal Depression Scale (EPDS), 11.4% of postpartum women scored above the threshold for depression. In similar studies, 18% of Australian women (Maloney, 1998), 8.3-12.5% of Swedish women (Wickberg & Hwang, 1997), 17 % of British women (Hearn et al., 1998) and 12% of Brazilian women were rated as depressed (Da-Silva, Moraes-Santos, Carvalho, Martins, & Teixeira, 1998) using the same instrument. Longitudinal studies suggest that the peak point prevalence for PPD is 10 weeks postpartum (Pop, Essed, de Geus, van Son, & Komproe, 1993).

It has been hypothesized that the etiology of postpartum affective disturbance is related to the dramatic fluctuations in hormone secretion and in biochemical parameters during the first days after childbirth. The increase in estradiol levels during pregnancy, followed by the rapid fall following parturition, has been a major focus of research. As with PMDD, studies that examined levels of estrogen and progesterone during the first week postpartum report conflicting results. Kuevi et al. (1983) found that blood levels of estrogen and progesterone tended to be lower in women with blues than in those without blues. However, Nott and associates (1976) assessed levels of progesterone and estrogen in women at two- or three-day intervals for up to ten weeks postpartum and found no significant differences between women who developed the blues and those who did not, although there was a correlation between irritability and higher pre-delivery estrogen levels. In a prospective study of the potential biologic and psychosocial factors associated with postpartum blues, an association between prepartum levels of estradiol and postpartum blues also occurred, implicating the larger *relative* drop in estrogen levels following parturition in the etiology of the postpartum blues. However, estradiol levels were not significantly correlated with measurements of the blues (O'Hara, Schlechte, Lewis, & Wright, 1991).

In contrast, several studies have reported a positive relationship between estrogen levels and the occurrence of the postpartum blues. For example, Feksi et al. (1984), measured salivary hormone levels four times daily for five days postpartum and found that women with higher maternity blues scores had higher levels of estrogen and progesterone than those with lower blues scores. In a similar study, women with

postpartum blues had significantly higher levels of total and free 17ß-estradiol when compared to women without postpartum blues, but hormone levels did not correlate with mood (Heidrich et al., 1994). Buckwalter and his associates (1999) also noted a positive association between decreases in estradiol and decreases in some measures of negative mood (specifically, the confusion and fatigue scales of the Profile of Mood States, and the Anxiety scale of the Symptom Check-List 90). Finally, other reports have failed to find a relationship between estrogen levels and postpartum blues during the first five days postpartum (Abou-Saleh, Ghubash, Karim, Krymski, & Bhai, 1998; Gard, Handley, Parsons, & Waldron, 1986).

In summary, just as with PMDD, there have been reports of positive relationships, negative relationships and the absence of relationships between estrogen levels and postpartum blues scores. The inconsistency in the literature may be the result of the wide variation in methodologies employed, such as sample sizes and inclusion criteria (e.g. lactation status), timing of blood samples, and hormonal assay techniques. More importantly, the marked variability in estrogen levels during the postpartum period may obscure any significant differences between groups, especially with small sample sizes. In fact, the problem of inter-subject variability in postpartum estrogen levels was noted by both Nott et al. (1976) and Kuevi et al. (1983) despite attempts to control for this factor.

While it has been difficult to demonstrate differences in estrogen levels between women who experience postpartum affective symptoms and those who do not, some

researchers have documented a beneficial effect of hormone replacement therapy on postpartum depression. For example, in a double-blind, placebo-controlled study of transdermal estradiol therapy for severe non-psychotic postpartum depression, the placebo and experimental groups showed significant differences in response to treatment (Gregoire, Kumar, Everitt, Henderson, & Studd, 1996). As measured by the Edinburgh Postnatal Depression Scale (EPDS), the estrogen-treated group showed a clinically and statistically significant improvement in depression within the first month of treatment as compared to placebo-treated women and this improvement was sustained for five months. Plasma estradiol concentrations were within the physiological range of menstrual cycle values in the estrogen group (mean value of 680 pmol/L at three months) and were significantly higher than in the placebo group. A similar positive response to treatment with exogenous estrogen occurred in a very small sample of women with treatment resistant postpartum depression and concomitant low levels of serum estradiol (Ahokas, Kaukoranta, & Aito, 1999). In addition, the administration of estrogen prophylactically has been shown to be effective in the prevention of recurrent postpartum affective disorder (Sichel, Cohen, Robertson, Ruttenberg, & Rosenbaum, 1995). High dose of oral estrogen (10 mg/day of Premarin) was administered to eleven women with a history of either puerperal psychosis or puerperal major depression immediately following delivery and was gradually tapered to follicular phase levels over a four-week period. Estrogen treatment prevented the recurrence of affective symptomatology in all but one patient.

In summary, while studies of the relationship between absolute levels of estrogen and mood in the postpartum period remain equivocal, there is clear evidence of increased

mood disturbance at this time, and this disturbance appears to be improved by treatment with exogenous estrogen.

Menopause:

Another time of significant hormonal fluctuation occurs in women with the gradual cessation of ovarian function associated with the menopause. During this time, follicular atresia and ovarian atrophy result in a decrease and eventual cessation of estrogen production (Longcope, 1990). This typically occurs at an average age of 51 in North American women (Miller & Franklin, 1999). In general, menopause refers to the last episode of menstrual bleeding, and it is commonly held that women are postmenopausal if they have not menstruated for one year (Carr & MacDonald, 1983; Miller & Franklin, 1999). The resultant estrogen deficiency may lead to a number of unpleasant physical symptoms, such as night sweats, cold sweats, hot flashes and hot flushes. All of these phenomena reflect "vasomotor instability" (Carr & MacDonald, 1983). Additional somatic symptoms of estrogen deficiency may include headaches, pains in the joints, and heart palpitations. The skin usually becomes drier. There is some change in breast tissue, thinning of the vaginal epithelium and decreased vaginal lubrication. Long-term estrogen deficiency has been linked to brittleness and porosity of the bones (osteoporosis) (DeCherney, 1993), and to an increased risk for cardiovascular disease (Sitruk-Ware, 1995).

Menopausal symptoms may also include various psychological complaints. Women in this age group receive more prescriptions for psychotropic drugs compared to

men of comparable age and to women in other age groups (Sator, Wieser, Gruber, Joura, & Huber, 1999; Skegg, Doll, & Perry, 1977; van der Waals, Mohrs, & Foets, 1993). In addition, changes in specific mood features have frequently been noted. Fifty year old women experience age-related peaks in the following symptoms compared with men of the same age: difficulty in making decisions, loss of confidence, anxiety, forgetfulness, difficulty in concentration, tiredness, and feelings of worthlessness (Bungay, Vessey, & McPherson, 1980). In a comparison of pre- and postmenopausal women, Beck depression inventory scores were shown to be significantly higher in postmenopausal women (Sagsoz, Oguzturk, Bayram, & Kamaci, 2001). Similarly, in a comparison of peri- and pre-menopausal women, significantly greater sleep disturbance (as measured by wrist movement monitors) and mood disturbance (as measured by the POMS) occurred in the peri-menopausal group (Baker, Simpson, & Dawson, 1997). In studies of patients attending menopause clinics, 65-86% of women reported depressive symptoms while up to 90% reported increased irritability (Anderson, Hamburger, Liu, & Rebar, 1987; Montgomery et al., 1987). Similar findings have also been reported cross-culturally, for example in Sri Lanka (Goonaratna, Fonseka, & Wijeywardene, 1999) and Thailand (Chompootweep, Tankeyoon, Yamarat, Poomsuwan, & Dusitsin, 1993).

Many postmenopausal women are prescribed hormone replacement therapy (HRT). In general, HRT for postmenopausal women with an intact uterus includes both estrogen and progestin (to reduce the risk of endometrial hyperplasia). There is evidence that such treatment improves mood and mood-related symptoms. Indirect evidence comes from a recent study that demonstrated an inverse relationship between the

prescription rate for HRT and the prescription rate for psychotropic drugs for menopausal women over the course of a five year period (1991-1996) (Sator et al., 1999). In addition, there is considerable direct evidence (specifically with respect to the role of estrogen). In a double-blind placebo controlled study of women with severe postmenopausal symptoms, Campbell (1976) demonstrated that 4 months of treatment with conjugated equine estrogen (1.25 mg daily) significantly improved ratings of insomnia, irritability, headaches, anxiety, urinary frequency, memory, good spirits, and optimism, as measured by a visual analogue rating scale. Dennerstein and colleagues (1979) also reported an improvement in symptoms of depression, as measured by the Hamilton Depression Rating Scale, in surgically menopausal women after treatment with estrogen (ethinyl oestradiol – 50 μg/day).

In a prospective, double-blind, placebo controlled, cross-over study of the impact of sex steroids on mood, Sherwin and Gelfand (1985), examined the effects of hormones administered individually or in combination in surgically menopausal women. Following the surgical removal of their uterus and ovaries for benign diseases, participants were randomly assigned to receive placebo, estrogen, androgen, or an estrogen-androgen combination. After three months, each subject was assigned to another treatment group. These investigators found that, in both treatment phases, the women who received a placebo had higher depression scores than those treated with any of the hormone preparations. This finding was replicated in another placebo controlled, cross-over investigation of the effects of intramuscular estrogen on mood in surgically menopausal women (Sherwin & Suranyi-Cadotte, 1990). Sherwin (1988) also compared surgically

menopausal women treated for an average of four years with an intramuscular estrogen-androgen combination, or estrogen alone, to a group who had been untreated after surgery. A positive correlation between ratings of confidence and elation and estrogen levels occurred in the treated women. These findings suggested that there is a positive association between plasma estradiol levels and mood in postmenopausal when values of estrogen are within the physiologic range.

In an attempt to control for the possibility that the mood-enhancing effect of estrogen occurred secondary to its ability to alleviate hot flushes, Ditkoff et al. (1991) conducted a randomized double-blind study of 36 asymptomatic and euthymic Hispanic American women, all of whom had undergone hysterectomy two years earlier. Conjugated equine estrogen (Premarin 0.625 mg or 1.25 mg/day) and a placebo were compared. There were small but significant decreases in depression scores for both estrogen-treated groups as measured by the Beck Depression Inventory (BDI), but no dose-dependent effect was evident.

Several recent studies have found estradiol treatment of depressed perimenopausal women to be effective in reducing symptoms. In a non-randomized study of clinically depressed postmenopausal women, treatment with conjugated equine estrogen (0.625 mg/day) for six months resulted in significant improvements in Hamilton depression rating scale scores compared to both baseline and to non-treated controls (Carranza-Lira & Valentino-Figueroa, 1999). Two recent randomized, double-blind, placebo-controlled studies of treatment with transdermal 17β-estradiol or placebo in

clinically depressed perimenopausal women found decreases in symptoms of depression after 3, 6 and 12 weeks of treatment with estrogen as compared to both baseline and placebo treated women. When placebo treated women were crossed-over to active treatment, their symptoms of depression were also improved (Schmidt et al., 2000). Partial or full remission of depression was noted in 68-80% of estrogen-treated women (Schmidt et al., 2000; Soares, Almeida, Joffe, & Cohen, 2001). In addition, it has been found that when estrogen is added to the antidepressant regimens of depressed postmenopausal women, treatment response is enhanced compared to placebo (Klaiber, Broverman, Vogel, & Kobayashi, 1979; Schneider et al., 1997).

In a large intervention trial designed primarily to examine the effects of various HRT regimens on cardiac functioning, no effect of estrogen on affect, cognition or anxiety were found (Greendale et al., 1998). However, these experimenters did not include a standardized measure of mood or of menopausal symptoms, making these data difficult to interpret.

Overall, the evidence is fairly consistent that estrogen treatment enhances mood in peri- and postmenopausal women. A recent comprehensive meta-analysis of the effects of HRT on depressed mood in menopausal women concluded that substantial and significant effects on mood were associated with estrogen treatment compared to placebo treatment (effect size = .73). Pretreatment vs. post-treatment comparison of mood scores also yielded significant effect sizes (effect size =.80) (Zweifel & O'Brien, 1997).

The Kindling Model of Depression:

It has been frequently noted that throughout the course of a recurrent major depressive disorder, depressive episodes show a pattern of becoming more autonomous and less linked to environmental events over time. This pattern of increasing autonomy has been referred to as the kindling model of depression (Kendler, Thornton, & Gardner, 2001; Post, 1992). The basic premise of kindling as it relates to affective disturbance is that the experience of each depressive episode reduces the threshold for an individual's neural circuitry to enter into a future depressive state. Eventually, the threshold is lowered to such an extent that episodes can occur spontaneously (without an obvious environmental stressor) (Kendler et al., 2001). These changes in the nervous system are believed to be essentially permanent (Weiss & Post, 1998).

Mood changes associated with the reproductive cycle may also sensitize the neuroendocrine system to future affective disorders (Halbreich, 2000; Parry, 1992, 1995). Similarly, an affective disorder may be exacerbated or precipitated during a reproductive event (Halbreich & Endicott, 1985). Prenatal depression has been shown to be predictive of postpartum depression (Buesching et al., 1986), premenstrual symptomatology has been linked to the development of severe maternity blues (Nott, Franklin, Armitage, & Gelder, 1976) and to the development of postpartum depression (Dennerstein, Morse, & Gotts, 1988) and a history of depression has been associated with severe premenstrual changes (Halbreich & Endicott, 1985). Moreover, patients with reproduction-related affective disturbance are at greater risk for depression during the menopause (Pearlstein, Rosen, & Stone, 1997). Therefore, a history of affective disturbance, particularly

reproduction-related affective disturbance, may contribute to an individual's vulnerability to environmental stressors and/or biological changes that may be associated with depressive affect.

Effects of Estrogen on Mood - Conclusion:

During pregnancy and during the late follicular phase of the menstrual cycle, when estrogen levels are high, mood is generally positive. Conversely, during the premenstrual phase of the menstrual cycle, the postpartum period, and at menopause, estrogen levels are declining, and depressed mood tends to occur with increased frequency. It seems clear, therefore, that the fluctuation of ovarian steroids during a variety of reproductive events might influence the emergence of affective symptomatology. Estrogens could exert their effects on mood via their effect on neurotransmitter systems (McEwen & Alves, 1999). More specifically, there is reason to believe that estrogen may interact with the serotonergic system - a system frequently implicated in depression.

Serotonin:

Serotonin and Depression:

While the exact role of serotonin metabolism in the pathophysiology of depression is still unclear, there none the less exists an impressive body of evidence supporting its involvement (Maes & Meltzer, 1995).

Studies of baseline differences of various measures of serotonin function in plasma and cerebrospinal fluid (CSF) between non-medicated depressed individuals and healthy controls have consistently demonstrated a link between the serotonin system and depression. For example, reduced CSF levels of the serotonin metabolite 5-HIAA (van Praag, 1977), and lower free plasma tryptophan (Coppen, Eccleston, & Peet, 1973), total plasma tryptophan (Cowen, Parry-Billings, & Newsholme, 1989) and CSF tryptophan (Coppen, Brooksbank, & Peet, 1972) were found in depressed individuals compared to healthy controls. There are also consistent reports of decreased blood platelet uptake of serotonin in depressed patients (Meltzer, Arora, Baber, & Tricou, 1981; Tuomisto & Tukiainen, 1976), and decreased numbers of ^{3}H-imipramine binding sites (presynaptic modulators of serotonin uptake thought to be a potential biological correlate of depression) (Paul, Rehavi, Skolnick, Ballenger, & Goodwin, 1981). These latter effects likely do not reflect trait differences in depressed individuals, but rather adaptive changes resulting from the depression (Arora & Meltzer, 1988). Some studies have also reported higher levels of 5-HT_{2A} receptors on the platelets of individuals with major depression who have attempted suicide (Mann, 1998). Finally, depressed patients with low CSF

levels of the serotonergic metabolite 5-hydroxyindole acetic acid (5-HIAA) have more frequent relapses than patients with normal levels of the metabolite, suggesting that low serotonin may predispose patients to depression (van Praag & de Haan, 1979).

Post-mortem studies of suicide victims have also provided evidence linking depression and serotonin. Several studies have found increased 5-HT_{2A} receptor binding sites in the frontal cortex of suicide victims (Arango et al., 1990; Arora & Meltzer, 1989; Stanley & Mann, 1983). The cause of the increase in the density of 5-HT_{2A} receptors is not completely understood, but may be related to a compensatory receptor up-regulation secondary to a presynaptic defect which results in lowered levels of neurotransmitter available to activate postsynaptic receptors (Arango et al., 1990).

Another strategy used to examine the role of serotonergic dysfunction in depression is through the use of challenge techniques. Using these techniques, changes in serotonin function may be assayed by measurement of the neuroendocrine response to infusion of substances known to affect serotonin function. One widely-used challenge agent is fenfluramine, which causes the release of serotonin and blocks its reuptake. This eventually leads to the release of prolactin from the pituitary into the bloodstream (Mann, 1999). Several fenfluramine challenge studies have shown a blunted prolactin response in depressed patients which suggests impaired serotonergic activity (Cleare, Murray, & O'Keane, 1996; Mann, McBride, Malone, DeMeo, & Keilp, 1995; O'Keane & Dinan, 1991).

Another frequently employed challenge technique examines the increase in prolactin secretion in response to tryptophan infusion in depressed and non-depressed individuals. Numerous tryptophan challenge studies of endogenously depressed patients have demonstrated blunting of the prolactin response (Cowen & Charig, 1987; Heninger, Charney, & Sternberg, 1984; Price, Charney, Delgado, & Heninger, 1991).

The drug parachlorophenylalanine has been used to experimentally deplete serotonin levels by selectively inhibiting its synthesis. Early serotonin depletion studies demonstrated that an experimental decrease in serotonin function induced by parachlorophenylalanine, resulted in symptomatic relapse in recently recovered depressed patients on antidepressant treatment. In contrast, no significant clinical effect was observed after administration of α-methyl-paratyrosine, an inhibitor of norepinephrine synthesis (Shopsin, Friedman, & Gershon, 1976; Shopsin, Gershon, Goldstein, Friedman, & Wilk, 1975) suggesting a role for serotonin in both the effects of antidepressant drugs and in the etiology of depression. More recently, studies examining the effects of acute tryptophan depletion (ATD) on mood have demonstrated findings consistent with a role for serotonin in depression. They are discussed in greater detail below.

Studies using functional brain-imaging techniques are making further important contributions in the attempt to determine the relationship between serotonin and depression. Using positron emission tomography (PET), comparisons of depressed individuals and healthy controls have revealed significant differences in 5-HT_2 receptor distribution (Biver et al., 1997), significantly decreased 5-HT_{1A} receptor binding

potential (Drevets et al., 1999; Sargent et al., 2000), blunted regional brain responses to serotonin release (Mann et al., 1996), and reductions in the density of brain serotonin transporter binding sites in living depressed patients (Malison et al., 1998).

Taken together, these findings suggest an etiological role for serotonin in the onset and maintenance of depression. As described below, estrogen has been shown to have a significant effect on the serotonergic system. Estrogen's effects on mood could therefore be mediated via the serotonergic system.

Mechanisms of Action of Estrogen on the Serotonergic System:

There is now a great deal of evidence that estrogen has profound and diverse effects on the serotonergic system (Fink, Sumner, Rosie, Grace, & Quinn, 1996; McEwen, 1994). The dorsal raphe nucleus is the largest single collection of serotonin-containing neurons in the brain (Jacobs & Azmitia, 1992). It also contains nuclear estrogen receptors (ER), as do the hypothalmus and limbic areas, which provides a potential mechanism whereby estrogen could alter serotonergic function (Bethea, Pecins-Thompson, Schutzer, Gundlah, & Lu, 1998). Recently, a new ER subtype (Erβ) was cloned from rat prostate (Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996). In humans, ERβ has a distinct mRNA distribution pattern from that of ERα. ERβ has been found predominantly in the hippocampal formation, entorhinal cortex, and thalamus whereas ERα mRNA has been found predominantly within the hypothalmus and amygdala (Osterlund, Gustafsson, Keller, & Hurd, 2000). Although the exact functions of

ERα and ERβ are not entirely known, the distribution patterns suggest that ERβ might be involved in memory and cognition, whereas ERα may be more important for estrogen's role in mood regulation (Osterlund et al., 2000).

The Metabolism of Serotonin (5-hydroxytryptamine):

In the CNS, the biosynthesis of serotonin requires two enzymatic steps. The precursor of serotonin, the amino acid L-tryptophan, is first hydroxylated by tryptophan hydroxylase to L-5-hydroxytryptophan (L-5-HTP). L-5-HTP is then decarboxylated to 5-hydroxytryptamine by a pyridoxal phosphate-dependent enzyme, aromatic amino acid decarboxylase. Monoamine oxidase converts 5-hydroxytryptamine to its metabolic end product, 5-HIAA, both in the neuron and in the synaptic space (Figure 1).

Estrogen can potentially affect the metabolic process of serotonin at the level of: 1) tryptophan (precursor) availability, 2) serotonin synthesis, 3) specific receptors, 4) the serotonin transporter (re-uptake), and 5) degradation of this neurotransmitter (via monoamine oxidase).

1) Tryptophan

Estrogen influences levels of the serotonin precursor L-tryptophan. Most of plasma tryptophan is bound to plasma albumin (McMenamy & Onclet, 1958). Estrogen competitively displaces some proportion of tryptophan from this binding site, resulting in an increase in the amount of unbound or "free" tryptophan in plasma, and therefore, an

increase in the amount of tryptophan available to the brain (Aylward, 1973). For example, a placebo controlled investigation of the effects of treating menopausal women with exogenous estrogen (oral estrone-piperazine sulphate, 3.0 mg/day) for three months demonstrated a significant increase in free plasma tryptophan in the treatment group. In addition, there was a significant positive correlation between plasma estrogen and free tryptophan levels following treatment (Aylward, 1976; Aylward & Maddock, 1973). Both of these findings have been replicated (Bender, Laing, Vale, Papadaki, & Pugh, 1983; Thomson, Maddock, Aylward, & Oswald, 1977). Therefore, estrogen can influence the serotonergic system by increasing the proportion of precursor available to the brain.

2) Serotonin synthesis

Estrogen alters the biosynthesis and release of serotonin (McEwen & Parsons, 1982; Oppenheim, 1983). More specifically, estrogen is thought to enhance serotonin synthesis (Bethea et al., 1998; Munaro, 1978; Shimizu & Bray, 1993). Administration of estradiol to ovariectomized rhesus macaques results in a ten-fold increase in tryptophan hydroxylase messenger RNA (mRNA) expression (Pecins-Thompson, Brown, Kohama, & Bethea, 1996) and a 4-6 fold increase in tryptophan hydroxylase protein expression (Bethea, Mirkes, Shively, & Adams, 2000) in serotonergic neurons of the dorsal raphe compared to ovariectomized controls. Since tryptophan hydroxylase is the rate-limiting enzyme in the synthesis of serotonin, the level of activity of this enzyme could play a role in the overall level of serotonin achieved (Bethea et al., 1998).

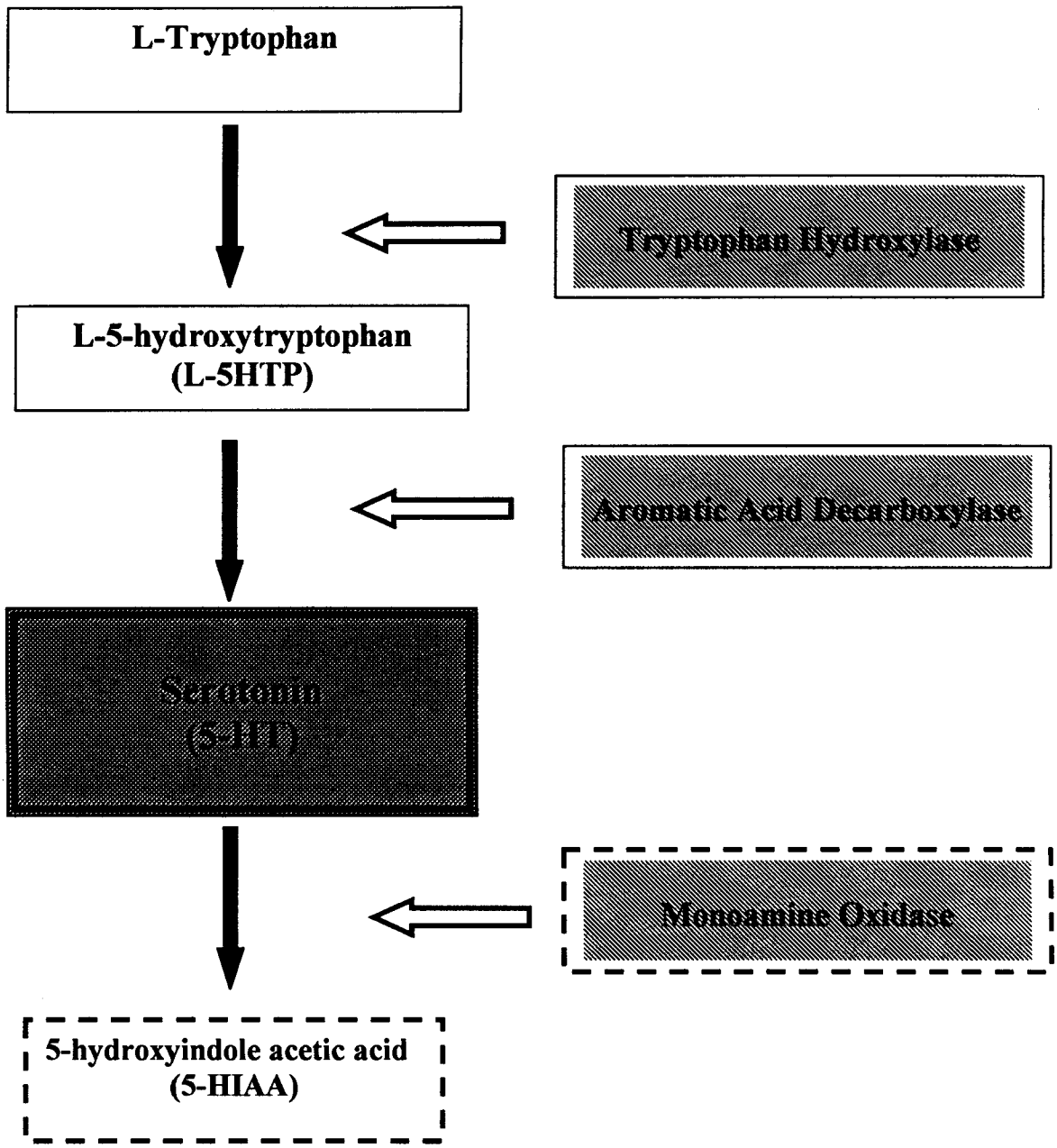


Figure 1: The Metabolism of Serotonin.

3) Specific Serotonin Receptors:

There are several serotonin receptor subtypes and, as biotechnology advances, new receptors are increasingly identified. Current classification systems group serotonin receptors into three general classes, each with several subtypes. These classes include 5-HT_1 receptors (which include autoreceptors), 5-HT_2 and 5-HT_3 receptors (Feldman, Meyer, & Quenzer, 1997). There have been several reports of an estrogenic influence on specific serotonin receptors. Estradiol treatment has stimulated a large increase in the expression of 5-HT_{2A} receptor mRNA in the dorsal raphe nucleus (Sumner & Fink, 1993). In addition, estradiol treatment in rats induced significant increases in the density of 5-HT_{2A} receptors in several regions of the brain including the cerebral cortex and nucleus accumbens (Cyr, Bosse, & Di Paolo, 1998; Fink & Sumner, 1996; Summer & Fink, 1995). Antidepressants have been shown to produce 5-HT_2 receptor down-regulation. Whereas ovariectomy prevents antidepressant-induced 5-HT_2 receptor down-regulation in the cerebral cortex in rats, the administration of estradiol restores the typical effects of an antidepressant on 5-HT_2 receptors (e.g. down regulation) (Kendall, Stancel, & Enna, 1981). These findings suggest that estrogen plays a role in the anti-depressant action of drugs that target the serotonin system.

The 5-HT_{1A} autoreceptor is of particular interest when examining the relationship between estrogen and the serotonergic system because of its inhibitory action on serotonergic activity. In an examination of the effects of ovarian steroids on 5-HT_{1A} mRNA using *in situ* hybridization, it was found that estrogen treatment of ovariectomized

macaques resulted in reduced dorsal raphe 5-HT_{1A} gene expression (Pecins-Thompson & Bethea, 1999). This would result in increased serotonin neurotransmission as a function of the reduction in the inhibitory effects of the serotonin autoreceptors.

4) Serotonin Transporter:

The primary means by which serotonergic neurons control extracellular levels of neurotransmitter is via the serotonin transporter (a plasma membrane carrier). This is accomplished by terminating the effects of synaptically released serotonin by means of the reuptake process. The Na^+ electrochemical gradient is the driving force for transport across the plasma membrane (Feldman et al., 1997). Estrogen appears to have a significant effect on the serotonin transporter although findings have not been entirely consistent in this respect. Using *in situ* hybridization, estrogen treatment of ovariectomized non-human primates significantly decreased serotonin transporter mRNA expression compared to untreated ovariectomized controls (Pecins-Thompson, Brown, & Bethea, 1998). The net result of this decrease is that serotonin remains in the extracellular space for a longer period of time in the presence of estrogen, thus prolonging neurotransmission (Bethea et al., 1998). In rats, however, the opposite result has been reported. Estradiol increased, by approximately 50%, the number of cells in the dorsal raphe nucleus that express serotonin transporter mRNA and increased serotonin transporter-binding sites by up to 250% (McQueen, Wilson, & Fink, 1997). Similarly, in rats, ovariectomy significantly decreased the density of presynaptic serotonin uptake sites which was reversed by the administration of exogenous estrogen. (Attali, Weizman, Gil-

Ad, & Rehavi, 1997). These inconsistencies may be explained by methodological differences, such as differences in species studied, the amount of time which lapsed following ovariectomy prior to estrogen treatment, and the duration of estrogen treatment.

5) Degradation of Serotonin:

It has long been known that estradiol can exert effects on monoamine oxidase (MAO), the enzyme that catabolizes serotonin (Klaiber, Broverman, Vogel, & Kobayashi, 1974). Estrogen decreases the activity of monoamine oxidase (Luine, Khylchevskaya, & McEwen, 1975) by increasing its rate of degradation (Luine & McEwen, 1977). The net effect of a lower concentration of the enzyme is increased levels of serotonin in the synapse.

Taken together these data provide strong evidence for a relationship between estrogen and the serotonergic system. Given that both estrogen and serotonin have been implicated in the regulation of mood, estrogen's actions on the serotonergic system presents several potential mechanisms of action for estrogen's role in reproduction-related mood changes.

Serotonin, Reproduction-Related Events, & Mood:

Menstrual Cycle:

Evidence suggests that serotonin may be important in the pathogenesis of PMDD (Steiner, 1997). For example, platelet MAO activity is decreased postmenstrually in PMDD patients compared to the premenstrual phase of the cycle (Ashby, Carr, Cook, Steptoe, & Franks, 1988). In addition, decreased uptake and lower levels of serotonin in platelets have been reported in PMDD participants premenstrually compared to postmenstrually (Taylor, Mathew, Ho, & Weinman, 1984) and in PMDD participants as compared to controls (Ashby et al., 1988). Rapkin et al. (1987) found reduced levels of serotonin during the luteal phase in patients with PMDD but this finding was not replicated in a later study by the same group, perhaps due to procedural differences (i.e., the fasting state of participants) (Rapkin, Chung, & Reading, 1989).

Much of the evidence regarding the relationship between the menstrual cycle and serotonergic function has come from neuroendocrine challenge studies using l-tryptophan, fenfluramine, or the 5-HT_{1A} agonist buspirone. For example, several studies have found significant effects of menstrual cycle phase and/or PMDD status in response to L-tryptophan challenge. Bancroft et al. (1991) administered intravenous tryptophan challenges to a group of participants with PMDD and to a healthy control group during the mid- to late-follicular phase, and, again during the late luteal phase of the menstrual cycle. They found that the typical increases in growth hormone and cortisol in response to tryptophan challenge were less marked in the PMDD group, compared to the control

group, on both occasions. The endocrine responses of participants with PMDD were similar to those seen in studies of patients with major affective disorders (Cowen & Charig, 1987). In addition, the prolactin response was blunted premenstrually compared to the mid-cycle phase in both the PMDD and control groups. These results suggest that a biochemical change occurs in all women premenstrually, comparable to the biological change that occurs in individuals with depressive illness, but it is not necessarily associated with premenstrual mood change unless other factors are also operating. These as yet undetermined factors may be associated with the abnormal cortisol and growth hormone responses observed in PMDD participants (Bancroft, Cook, Davidson, Bennie, & Goodwin, 1991). Rapkin and her associates (1989) also demonstrated a difference between healthy control participants and PMDD patients in their whole-blood serotonin response to an oral L-tryptophan load. Whole-blood serotonin levels are thought to predict brain serotonin levels (Raleigh et al., 1980). A consistent increase in whole-blood levels of serotonin was observed in control participants at all sampling intervals across the menstrual cycle. In PMDD patients, however, whole-blood serotonin levels were increased in the follicular and mid-luteal phases, but decreased during the late luteal phase. The responses of the PMDD participants to tryptophan challenge in the late luteal phase were significantly different from their responses in both the mid-luteal and follicular phase (i.e. whole blood serotonin levels were decreased as opposed to increased). In comparison to controls, the difference was significant only during the late luteal phase. These results imply that women with PMDD metabolize tryptophan differently in the late luteal phase, and lend support to the idea that there is a deficit in the serotonin system of PMDD sufferers during the premenstrual phase of the cycle (Rapkin,

Chang, & Reading, 1989). This finding was recently replicated using IV (as opposed to oral) infusions of tryptophan (Rasgon, McGuire, Tanavoli, Fairbanks, & Rapkin, 2000), although the generalized blunted cortisol response to tryptophan in women with PMDD and the blunted late luteal prolactin response in both healthy women and those with PMDD found by others (Bancroft et al., 1991) was not confirmed (Rasgon et al., 2000). Studies using fenfluramine (a serotonin agonist) as a challenge agent have revealed both positive (FitzGerald et al., 1997) and negative (Steiner, 1997) results with respect to premenstrual blunting of the prolactin response in women with PMDD. In the latter study, however, other evidence of serotonergic dysfunction in women with PMDD was noted, including a delayed response to fenfluramine at both phases of the menstrual cycle and decreased ^{3}H-imipramine binding levels during the luteal phase compared both to controls and to the follicular phase responses.

Investigations of serotonergic functioning in healthy females using the 5-HT_{1A} agonist, buspirone, have also found evidence of menstrual cycle-linked changes in response to experimental challenge (Dinan, Barry, Yatham, Mobayed, & O'Hanlon, 1990; Yatham, Barry, & Dinan, 1989). For example, the prolactin response to buspirone was increased during the luteal phase, relative to the follicular and midcycle phases (Dinan et al., 1990). This indicates supersensitivity of 5-HT_{1A} receptors when estrogen and progesterone levels are decreasing. In contrast, male participants showed consistency in their response to buspirone over repeated testing (Dinan et al., 1990). The fact that the prolactin response was enhanced premenstrually in healthy women, rather than blunted as

was the case after L-tryptophan challenge in healthy women (Bancroft et al., 1991) may be explained by the specificity of the action of buspirone on 5-HT_{1A} receptors.

Given these findings on the possible role of serotonergic dysfunction in PMDD, researchers turned their attention to examining the potential treatment benefits of serotonin agonists. Several randomized, double-blind, placebo-controlled trials of the efficacy of the selective serotonin re-uptake inhibitor (SSRI), fluoxetine, in the treatment of PMDD have shown it to be significantly superior to placebo in reducing both the physical and emotional symptoms associated with the syndrome (Steiner et al., 1995; Su et al., 1997). A double-blind, placebo-controlled pilot study of the 5-HT_{1A} agonist, buspirone, also found a significantly greater decrease in PMDD symptoms compared to placebo (Rickels, Freeman, & Sondheimer, 1989). In addition, the results of a recent comparison of the efficacy of another SSRI (sertraline), of a tricyclic, noradrenergic antidepressant (desipramine) and of placebo found that the serotonergic drug was significantly more effective in the treatment of PMDD. Interestingly, the noradrenergic drug was not superior to placebo on any measure of mood or quality of life in that comparison study (Freeman, Rickels, Sondheimer, & Polansky, 1999).

Postpartum:

There is less information on the role of serotonergic functioning in postpartum mood changes. In an investigation of the relationship between serotonin metabolism and dysphoria during the postpartum period, several significant correlations were reported. Mean Michaelis-Menton constant (Km), a measure of serotonin uptake, was significantly

reduced in the group showing dysphoria at 5 days postpartum as measured by the EPDS. In addition, the mean dissociation constant (Kd) of ^{3}H-imipramine, a measure of binding affinity, was significantly increased in the group who later went on to become depressed at 6 weeks. No significant differences were found with respect to MAO activity (Hannah, Adams, Glover, & Sandler, 1992). These findings suggest that the biochemical abnormalities associated with mood changes during the postpartum period are different from those seen in affective disturbance in general. Despite these differences, a treatment study found beneficial effects of SSRIs compared to placebo in women with PPD (Appleby, Warner, Whitton, & Faragher, 1997).

Menopause:

There is an increasing amount of evidence in support of the idea that changes in serotonergic function occur perimenopausally, and may be reversed by estrogen replacement therapy (ERT). In a study examining the effect of ovarian function on whole-blood serotonin in menopausal women, naturally postmenopausal women had lower blood serotonin levels than regularly menstruating women (Gonzales & Carrillo, 1993). Following estrogen replacement therapy, blood serotonin levels increased to values similar to those observed in young, regularly menstruating women. In addition, there was a significant positive relationship between serum estradiol and blood serotonin levels. In another study of 12 postmenopausal women, low levels of plasma serotonin were found, but only a trend for reversal of this effect with ERT occurred (Blum et al., 1996).

In a double-blind, cross-over investigation of surgically menopausal women, an increase in the number of ^{3}H-imipramine binding sites on platelets and an improvement in mood occurred following treatment with exogenous estrogen (Sherwin & Suranyi-Cadotte, 1990). These effects disappeared in the cross-over portion of the study when placebo was substituted for estrogen. In addition, treatment of menopausal women with estrogen caused a significant increase in urinary excretion of 5-HIAA which is thought to reflect an increase in serotonin metabolism (Lippert, Filshie, Muck, Seeger, & Zwirner, 1996; Mueck, Seeger, Kasspohl-Butz, Teichmann, & Lippert, 1997).

Results of serotonin challenge studies of postmenopausal women have been consistent with the notion of a postmenopausal serotonergic dysfunction. For example, a blunted prolactin response to the serotonin agonist meta-chlorophenylpiperazine (m-CPP) occurred in menopausal women compared to regularly cycling women (Halbreich et al., 1995). Following ERT, the prolactin response was increased in the postmenopausal women.

Taken together, the above findings on the relationship between serotonin and the menstrual cycle, the postpartum period and the menopause strongly suggest that higher estrogen levels are associated with higher levels of serotonin and implicate the serotonergic system in estrogen's action on mood.

Tryptophan:

Tryptophan and Serotonin:

Because serotonin does not cross the blood brain barrier, serotonin levels in brain are dependent on local conversion of the large neutral amino acid (LNAA) L-tryptophan to serotonin (Fernstrom & Wurtman, 1971). Other large neutral amino acids (e.g., tyrosine, valine, leucine, isoleucine, and phenylalanine) compete for the same carrier protein for transport across the blood-brain barrier (Oldendorf & Szabo, 1976). As a result, the brain level of each amino acid depends not only on the level of that amino acid in the plasma, but also on the plasma levels of the other amino acids competing for the transport system. Hence, the ratio of tryptophan to other LNAAs must be high before it is able to cross the blood brain barrier and enter the brain.

Tryptophan and Depression:

Studies of tryptophan metabolism in depressed and normal participants have found decreased basal levels of free and total tryptophan in depressed individuals (Coppen & Wood, 1978; Cowen et al., 1989; DeMyer, Shea, Hendrie, & Yoshimura, 1981). In addition, decreased basal ratios of plasma tryptophan to the other LNAAs also occurs in depressed patients (Cowen et al., 1989; DeMyer et al., 1981). Another noteworthy finding is that of an inverse correlation between scores on the Hamilton Depression Rating Scale and the ratio of plasma tryptophan to the other LNAAs (DeMyer et al., 1981). Depressed patients who responded positively to serotonin re-uptake

inhibitors had a lower pre-treatment plasma-tryptophan/LNAA ratio (Lucca, Lucini, Catalano, Alfano, & Smeraldi, 1994). The evidence for a lower tryptophan/LNAA ratio in depressed individuals has not been entirely consistent since others found that total and free plasma tryptophan to LNAA ratios were significantly lower in depressed participants only after L-tryptophan loading, but not after placebo, in both the acute and the partial recovery phase (Russ, Ackerman, Banay-Schwartz, Shindledecker, & Smith, 1990).

Since tryptophan crosses the blood brain barrier, and because tryptophan hydroxylase is unsaturated and restricted to serotonin neurons, oral administration of tryptophan gives rise to selective increases in serotonin synthesis and release (Fernstrom & Wurtman, 1971; Wurtman, 1982). Therefore, L-tryptophan supplementation has been used in the management of several CNS disorders associated with decreased serotonin function, including depression (Young, 1991). In depressed patients, 5-hydroxytryptophan (L-5HTP) may have an antidepressant action (van Praag, 1981), and intravenous administration of L-tryptophan significantly improves mood in healthy individuals (Charney, Heninger, Reinhard, Sternberg, & Hafstead, 1982).

Tryptophan, Reproduction-Related Events, & Mood:

Tryptophan has also been implicated in reproduction-related mood changes. As discussed earlier, one way that estrogen might exert an influence on the serotonergic system is by increasing the concentration of tryptophan available to the brain, which is then synthesized to serotonin.

Menstrual Cycle:

The rate of tryptophan metabolism appears to vary across the menstrual cycle. An increased catabolism of peripheral tryptophan via the kynurenine pathway occurs in healthy women in the luteal phase compared with the follicular phase of the menstrual cycle (Hrboticky, Leiter, & Anderson, 1989). After tryptophan loading, a 40% increase in plasma concentration and a 28% increase in 24-hour urinary kynurenine levels occurs in the luteal, relative to the follicular, phase of the cycle. These findings imply that there is a decrease in serotonin synthesis during the luteal phase (Hrboticky et al., 1989). However, neither free nor total plasma tryptophan levels differ significantly between pre- and post-menstrual phases of the menstrual cycle or between PMDD patients and controls (Ashby et al., 1988). In a similar investigation of the plasma tryptophan/LNAA ratios in women with PMDD and control participants, no significant differences were seen between groups or between phases of the menstrual cycle (Rapkin et al., 1991). The report that the ratio of tryptophan to other LNAAs in plasma does not differ significantly between the follicular, mid-cycle, or late luteal phases in healthy drug-free women has been confirmed, as has the report that there are no significant fluctuations in plasma tryptophan across the menstrual cycle (Moller, Maach-Moller, Olesen, & Fjalland, 1993).

It is possible that fluctuations in plasma levels of tryptophan and tryptophan/LNAA ratios across the menstrual cycle cannot be demonstrated in patients with PMDD due to selective increases in carbohydrate intake during the luteal phase of the menstrual cycle (Rapkin et al., 1991). Carbohydrate craving can be perceived as a

means of self-medication because of their effect on mood via their influence on serotonin concentrations (Christensen & Redig, 1993). Carbohydrate-rich diets increase brain L-tryptophan levels in rats while protein-rich diets decrease it (Fernstrom & Faller, 1978). The consumption of a carbohydrate-rich, protein-poor meal results in increased central serotonin synthesis and release due to its effect on the plasma ratio of tryptophan to the other LNAAs. More specifically, after the consumption of a carbohydrate-rich, protein poor meal, insulin is secreted which results in the net uptake of the branched-chain amino acids (leucine, isoleucine, and valine) into muscle as well as a dissociation of nonesterified fatty acid molecules from albumin. Tryptophan is spared, however, because it binds loosely to the available albumin that has been stripped of the fatty acid molecules. As a result, the plasma levels of the branched chain amino acids decline and the plasma tryptophan/LNAA ratio increases. As this ratio becomes more heavily weighted in favor of tryptophan, the amount of tryptophan that crosses the blood-brain barrier increases. This results in an increase in the saturation of the enzyme tryptophan hydroxylase, which in turn increases the rate of serotonin synthesis (Wurtman, 1987).

Support for the hypothesis that increased premenstrual carbohydrate intake could serve to improve mood is derived from a study of women with PMDD in which the consumption of a carbohydrate-rich, protein-poor test meal during the late luteal phase of the menstrual cycle significantly reduced depression, tension, anger, confusion, and fatigue as measured by the Profile of Mood States (Wurtman, Brzezinski, Wurtman, & Laferrere, 1989). No effect of the meal on mood was observed in PMDD participants during the asymptomatic follicular phase of the cycle or among the control participants

during either cycle phase. Despite the fact that the amino acid ratios among the women with premenstrual symptoms were similar during the follicular and luteal phases, mood enhancement occurred only during the luteal phase, suggesting a therapeutic effect of carbohydrate-rich foods during the late luteal phase in patients with PMDD (Wurtman et al., 1989). It has also been suggested that nutrient intake naturally fluctuates over the menstrual cycle in women with PMDD. For example, participants with severe PMDD consumed significantly more calories and demonstrated a specific preference for carbohydrate-rich foods during the luteal phase of the menstrual cycle compared to control participants during that phase (Wurtman et al., 1989). No food preferences were seen during the follicular phase of the cycle for either group. In contrast, a similar study found that the total caloric intake and the proportion of carbohydrates to protein in the meals consumed by healthy females also varied during the menstrual cycle, with increased levels during the luteal, relative to the follicular, phase (Bowen & Grunberg, 1990). The inconsistency between these two studies is likely explained by the rigid criteria used to define both PMDD participants (severe symptoms) and non-PMDD participants (no symptoms at all) in the study by Wurtman et al. (1989). The healthy participants in the Bowen and Grundberg (1990) study experienced some premenstrual mood changes, making it more likely that they would also experience changes in food intake. Therefore, while it has been difficult to demonstrate fluctuations in plasma tryptophan levels across the menstrual cycle (Ashby et al., 1988; Moller et al., 1993; Rapkin et al., 1991), inadequate control over nutrient intake may have confounded the results of these studies.

Only two groups have investigated the potential therapeutic effects of tryptophan in PMDD. In the first, relatively low doses of L-tryptophan (mean=3.7 grams) in combination with pyridoxine (vitamin B6) was not more effective than placebo in controlling symptoms of PMDD, and resulted in significant side-effects (Harrison, Endicott, Rabkin, & Nee, 1984). In a more recent investigation, however, the administration of 6 grams of L-tryptophan alone to women with PMDD was associated with a significant improvement of symptoms of depression, mood swings and irritability with only mild side effects. These findings suggest that the potentiation of serotonergic functioning may be important in the treatment of PMDD and that this may be accomplished by administration of exogenous tryptophan (Steinberg, Annable, Young, & Liyanage, 1999).

Postpartum:

The role of tryptophan in postpartum depression and blues has been examined quite extensively (Handley, Dunn, Baker, Cockshott, & Gould, 1977) (Stein, Milton, Bebbington, Wood, & Coppen, 1976). For example, Stein et al. (1976) measured plasma tryptophan levels in eighteen postpartum women and correlated them with daily measures of mood for seven to eight days following parturition. A significant negative correlation occurred between severity of affective changes and free tryptophan, and levels of free tryptophan were similar to those in a group of depressed non-postpartum women. These observations were confirmed by Handley et al. (1977), who also found a significant inverse correlation between free plasma tryptophan and depression during the postpartum period. In an extension and replication of this study with a much larger group of

postpartum women, free tryptophan was reduced in blues participants only at the time of year when, according to the authors, plasma free tryptophan is normally high (June to December) (Handley, Dunn, Waldron, & Baker, 1980). In addition, the absence of the typical postpartum peak in total tryptophan on day one or two postpartum was significantly related to occurrence of postpartum blues and complaints of depression in the ensuing 6 months. A subsequent multifactorial study also found that a slower rise in total tryptophan predicted the occurrence of postpartum blues (Gard et al., 1986). A low blood level of tryptophan on day one postpartum was particularly predictive of subsequent development of postpartum blues. Finally, in a comparison of tryptophan levels during the postpartum period to those observed during the course of oral contraceptive therapy and to naturally cycling controls, plasma tryptophan levels were lower in the postpartum period while the tryptophan/LNAA ratio did not differ across groups. In addition, the absence of a postpartum peak of total tryptophan predicting blues was confirmed, and a significant negative correlation between the tryptophan /LNAA ratio and anxiety was found (Maes et al., 1992).

Although the above findings suggest that the administration of exogenous tryptophan might help to prevent postpartum blues, a prospective double blind trial of tryptophan versus placebo in 55 postpartum women failed to show a significant reduction in blues symptoms in (Harris, 1980). Therefore, while there does appear to be a relationship between tryptophan levels and postpartum mood changes, there is no evidence to date that administration of exogenous tryptophan can prevent them.

Menopause:

Far less research has focused on the role of tryptophan in the mood changes associated with the menopause, although there does appear to be a relationship between estrogen and tryptophan levels at this time. A positive correlation has been reported between total plasma estrogen and free plasma tryptophan in postmenopausal women (Aylward, 1976; Aylward & Maddock, 1973; Thomson et al., 1977). In a randomized, double-blind, placebo-controlled study, the administration of exogenous estrogen (estrone-piperazine sulfate 3.0 mg/day) to postmenopausal women resulted in a significant increase in plasma free tryptophan compared to control participants (Aylward & Maddock, 1973). As well, a highly significant positive correlation between improvement in depressive symptoms and free plasma-tryptophan concentrations occurred (Aylward & Maddock, 1973). In a similar study, administration of exogenous estrogen (ethinyl oestradiol 20 μg/day or piperazine oestrone sulfate 3 mg/day) to peri- and postmenopausal women for three months resulted in an increase in the concentration of total tryptophan in plasma, while free tryptophan was increased significantly only in women who received ethinyl oestradiol (Bender et al., 1983). A significant positive relationship between increases in plasma concentrations of tryptophan and improvement of depressive symptoms also occurred.

Overall, these data suggest a positive relationship between estrogen levels and tryptophan levels, as well as a positive relationship between tryptophan levels and mood during the menopause.

Acute Tryptophan Depletion:

While there is a substantial body of evidence implicating deficits in both serotonin and tryptophan in the process of mood regulation, it is largely correlational in nature. This is in part due to ethical constraints associated with the artificial manipulation of brain function in humans. For this reason, a considerable amount of recent research has focused on the use of dietary strategies which are short-lived, non-toxic, and which make use of normal metabolic processes to experimentally alter brain function. The acute tryptophan depletion technique requires that participants ingest a mixture of amino acids designed to lower brain serotonin levels. In the most commonly used tryptophan deficient and nutritionally balanced mixtures, the amino acids are mixed in the same proportions as they occur in human milk and are therefore suitable for use with human participants (Young, Ervin, Pihl, & Finn, 1989).

Effects of Acute Tryptophan Depletion on the Serotonergic System:

Animal studies examining tryptophan depletion have demonstrated that the administration of a diet devoid of tryptophan results in a marked decrease in total and free serum tryptophan levels as well as significant decreases in tryptophan, serotonin and 5-HIAA levels in the brain. These changes occur 2 hours after ingestion of the tryptophan-deficient meal and are maintained for 24 hours (Biggio, Fadda, Fanni, Tagliamonte, & Gessa, 1974). In order for treatment-induced changes in plasma and brain precursor levels to cause parallel alterations in the synthesis of their

neurotransmitter products, the enzyme catalyzing the key step in this biotransformation must be capable of generating more product when exposed to increased concentrations of its substrate. In the case of serotonin, this requires that the enzyme tryptophan hydroxylase (the rate-limiting enzyme of the tryptophan to serotonin biosynthetic pathway) not be fully saturated with the precursor (tryptophan). That is, the affinity of the enzyme for its substrate must be poor in relation to available substrate concentrations (Wurtman, Hefti, & Melamed, 1981). Because tryptophan hydroxylase is normally only approximately 50% saturated with tryptophan (Fernstrom & Wurtman, 1971), a diet devoid of tryptophan results in a marked lowering of brain serotonin, in addition to decreases in blood and tissue serotonin (Biggio et al., 1974). In rats, a decline in total plasma tryptophan of 75% causes brain serotonin to drop to about 50% of control values (Biggio et al., 1974). Recent *in vivo* brain microdialysis studies of freely moving rats replicated and extended these findings using amino mixtures similar in composition to those used in human studies. They demonstrated decreases in serotonin and 5-HIAA concentrations in brain following oral administration of a tryptotphan-deficient amino acid mixture as well as decreases in serotonin release following treatment with fluvoxamine or d-fenfluramine (Bel & Artigas, 1996; Stancampiano et al., 1997). Similarly, in vervet monkeys, ingestion of tryptophan-free amino acid mixtures causes significant decreases in plasma and CSF tryptophan levels, as well as significant decreases in CSF levels of 5-HIAA. It is noteworthy that these decreases occur in the absence of changes in CSF tyrosine levels, or in CSF levels of the catecholamine metabolites homovanillic acid (HVA), or 3-methoxy-4-hydroxyphenylethylene glycol levels (MHPG). This indicates that the effect of tryptophan depletion is specific to the

serotonin system, and provides evidence for the hypothesis that the behavioral effects of tryptophan depletion are mediated by a decline in serotonin (Young et al., 1989).

In humans, a 100 g mixture of amino acids devoid of tryptophan causes a 70% to 90% decline in plasma tryptophan (Young et al., 1989). Plasma tryptophan reaches its lowest level approximately five hours after ingestion of a tryptophan-deplete mixture, and it remains low for several hours (Young, Smith, Pihl, & Ervin, 1985). There is also convincing evidence that brain serotonin is reduced in humans using the acute tryptophan depletion technique (Young, 1993). The finding that brain tryptophan hydroxylase is also only approximately half saturated with tryptophan in humans (Young & Gauthier, 1981) extends findings from the animal literature (Fernstrom & Wurtman, 1971). Therefore, the decline in the availability of tryptophan produced by tryptophan depletion should cause a similar lowering of brain tryptophan in humans as in rats. That this occurs was demonstrated by a PET study which found a highly significant reduction in the rate of serotonin synthesis five hours after acute tryptophan depletion (Nishizawa et al., 1997). In addition a significant lowering of CSF 5-HIAA has been observed 4 to 14 hours following administration of the tryptophan deficient drink in humans, providing further evidence for decreased serotonin synthesis and metabolism following tryptophan depletion (Carpenter et al., 1998; Williams, Shoaf, Hommer, Rawlings, & Linnoila, 1999).

In an investigation of the mechanisms underlying the decrease in serotonin following tryptophan depletion, it was concluded that the decrease in plasma tryptophan

occurs because the tryptophan deficient amino acid mixture, like any mixture of essential amino acids, promotes synthesis of new protein. The tryptophan that is incorporated into this protein comes from tryptophan pools in blood and tissues, and therefore its levels in plasma and brain falls (Gessa, Biggio, Fadda, Corsini, & Tagliamonte, 1974). The importance of protein-synthesis as a mechanism by which serotonin is lowered was confirmed by evidence that the depletion of tryptophan could be blocked by a protein-synthesis inhibitor (Moja et al., 1991)

Another mechanism might contribute to the decline in brain (but not plasma) tryptophan. All the large neutral amino acids (LNAA) compete for the same carrier system which transports them across the blood-brain barrier (Oldendorf & Szabo, 1976). Therefore brain tryptophan levels depend not only on plasma tryptophan, but also on the plasma levels of the other LNAAs (Fernstrom & Wurtman, 1972). Ingestion of a tryptophan-deficient mixture would cause a significant decrease in the ratio of tryptophan to the other LNAAs, resulting in significantly less tryptophan crossing the blood-brain barrier.

Tryptophan Depletion and Mood:

Studies of the behavioral impact of tryptophan depletion in humans have found a significant effect on mood. For example, in healthy males whose baseline depression scores were in the high-normal range, the tryptophan deplete mixture caused a significant elevation in depression scores and a significant decrease in concentration as measured by

the Multiple Affect Adjective Checklist (MAACL). However, neither the anxiety nor hostility scales of the MAACL were significantly affected, demonstrating a specificity of the tryptophan depletion effect on mood. Because participants in this study scored in the high-normal range on depression scales at pretreatment, questions remained regarding the effects of acute tryptophan depletion on euthymic participants with normal depression-scale scores. It was subsequently found that acute tryptophan depletion in participants with low baseline depression scale scores caused no change in POMS scores (Abbott et al., 1992). Thus, it may be the case that euthymic individuals are not sensitive to the mood altering properties of acute tryptophan depletion.

The effects of tryptophan depletion on clinically depressed individuals have resulted in several interesting findings. Patients with major depressive disorder (MDD) successfully treated with antidepressant drugs and patients with seasonal affective disorder successfully treated with bright light at the time of testing showed a rapid reappearance of the full syndrome 5-7 hours following acute tryptophan depletion (Delgado et al., 1990; Delgado et al., 1999; Lam et al., 1996; Neumeister et al., 1997; Spillmann et al., 2001). However, untreated depressed patients showed no change in mood in response to tryptophan depletion. This may have been due to a floor effect of the severity of the participants' depression (Delgado et al., 1994).

Thus, there is evidence to suggest that the degree of mood-lowering after tryptophan depletion is related to the baseline mood state of the participant as well as to the biological vulnerability of the participant to depression. In order to test this

hypothesis, the effects of acute tryptophan depletion were compared in healthy male participants genetically predisposed to develop MDD and control participants with no such predisposition (Benkelfat, Ellenbogen, Dean, Palmour, & Young, 1994). Participants with a family history of MDD showed a significantly greater increase in POMS depression scale scores following tryptophan depletion than the group with a negative family history of MDD. This difference occurred despite the fact that all the participants had normal baseline POMS depression scale scores that did not differ between groups. These results are consistent with the idea that the degree of mood-lowering after acute tryptophan depletion is related to the participant's susceptibility to depression and have been replicated (Klaassen, Riedel, van Someren et al., 1999). Also consistent with this concept is the finding that untreated, remitted depressed individuals show a significantly greater depressive response to tryptophan depletion than healthy controls (Moreno et al., 1999), but this finding has not been entirely consistent (Leyton et al., 1997).

In healthy women, acute tryptophan depletion causes a significant lowering of total and free tryptophan within approximately 4.5 hours (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1996; Oldman, Walsh, Salkovskis, Laver, & Cowen, 1994; Wolfe, Metzger, & Jimerson, 1995). Inconsistent results have been reported with respect to the mood effects of acute tryptophan depletion in women. In women with a history of major depressive disorder, acute tryptophan depletion significantly lowers mood as measured by the POMS compared to administration of a balanced amino acid drink (Smith, Fairburn, & Cowen, 1997, 1999), but in women with a multigenerational family history

of major affective disturbance, no effect of tryptophan depletion was reported (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1999). A significant lowering of mood has been demonstrated in some examinations of healthy women following acute tryptophan depletion (Ellenbogen et al., 1996; Klaassen, Riedel, Deutz, van Someren, & van Praag, 1999; Leyton et al., 2000) but not in others (Oldman et al., 1994; Smith et al., 1999). One possible explanation for these inconsistencies may relate to the fact that all studies to date have examined premenopausal women and therefore results may have been influenced by menstrual cycle linked hormonal changes. While some studies attempted to control for phase of cycle by limiting tests days to the follicular phase of the menstrual cycle, phase of cycle was determined largely by self-report and was not confirmed with objective measures of hormone levels. Given that even within a menstrual cycle phase there is a significant amount of hormonal fluctuation (e.g. from the early to late follicular phase, estrogen levels increase substantially), these types of controls may not have been sufficient.

Tryptophan Depletion, Reproduction-Related Events, & Mood:

The influence of the serotonergic system in PMDD was tested by the administration of the acute tryptophan depletion technique at two different phases of the menstrual cycle in 16 participants with well-documented PMDD. An increase in symptoms of PMDD occurred following acute tryptophan depletion during the luteal phase of the menstrual cycle, and, to a lesser degree during the follicular phase of the menstrual cycle (Menkes, Coates, & Fawcett, 1994). While these results are interesting,

they must be interpreted with caution for several reasons. First, both untreated women and those who were currently obtaining partial symptomatic relief from fluoxetine (4 participants) or oral contraceptives (3 participants) were included. It is likely that these treatments would have affected ovarian function and/or behavioral responses to acute tryptophan depletion. Of the 16 women, cycle phase was confirmed in only 11 by menstrual records and plasma progesterone assays. Finally, the use of only one mood scale designed to specifically assess premenstrual symptoms makes comparison to other studies difficult. Moreover, the severity of premenstrual symptomatology experienced by these participants makes it difficult to generalize these findings to healthy women experiencing mild fluctuations in mood associated with normal alterations in reproductive hormones. This is especially true given the fact that estrogen levels were not measured, thereby making comparison impossible.

Hypotheses:

In order to further elucidate the role of estrogen in the regulation of mood, the present study examined the interplay between estrogen level and acute tryptophan depletion. Using postmenopausal women as an experimental model of low endogenous estrogen, the mood-lowering effect of acute tryptophan depletion was compared in participants randomly treated with exogenous estrogen and those receiving placebo treatment. The following outcomes were postulated:

I) Overall, postmenopausal women would be vulnerable to the mood lowering effects of acute tryptophan depletion. If that occurred, it would provide corroborating evidence that female sex is a vulnerability factor to acute tryptophan depletion as has been previously suggested (Ellenbogen et al., 1996).

II) Treatment with exogenous estrogen would contribute to maintaining a euthymic mood state in postmenopausal women, thereby providing some protection against the mood-lowering effects of the acute tryptophan depletion procedure (Abbott et al., 1992). If that occurred, it might suggest that a low estrogen state is a vulnerability factor for dysphoric mood changes in women.

III) A sub-sample of women who were more susceptible, or sensitive, to the effects of estrogen on mood would be identified. Those women who were sensitive to the mood enhancing effects of exogenous estrogen would benefit to a greater degree from estrogen's postulated protective effects following acute tryptophan depletion than those women who were less sensitive to estrogen's overall mood effects.

IV) A past history of affective disturbance, particularly reproduction-related affective disturbance such as PMDD or PPD, would be predictive of a more profound response to acute tryptophan depletion. If this occurred, it would provide support for the kindling model of depression which postulates that prior experience with a reproductive-related affective disorder sensitizes women to future depressive episodes in response to environmental or physiological stressors (Halbreich, 2000; Parry, 1992, 1995).

METHODS

Participants:

Participants were recruited through advertisements in a city newspaper, The Montreal Gazette and other smaller, local newspapers (e.g. The Suburban, The Reporter) seeking menopausal women (aged 40-60) not currently receiving hormone replacement therapy to participate in a study examining the influence of hormones and dietary factors on mood in women (see Appendix 1).

Six hundred and sixty-nine women responded to the newspaper advertisements and underwent preliminary phone screening (10-30 minutes). Women who were not yet menopausal, were currently taking hormones, were currently taking any psychotropic medications or other medications with possible CNS effects (including beta blockers and some analgesics), were currently being treated for psychological disorders, had any serious acute or chronic medical conditions (including diabetes, coronary heart disease and neurological disorders), or who had a personal or family history of breast cancer were excluded. Women with thyroid disease that was controlled with medication (synthroid) were not excluded. Women who reported obvious symptoms of psychopathology such as major affective disturbance or suicidal ideation were offered referrals to appropriate clinicians or clinics in their area.

Women who were still eligible to participate in the study following the telephone screening were provided with a brief description of the nature and purpose of the study over the telephone and offered the opportunity to schedule an appointment for an initial interview and baseline evaluation. Of those women who were eligible, 126 agreed to participate.

The majority of women who qualified for participation but chose not to do so after the telephone screening cited concern about receiving estrogen or any form of medication as their primary reason. Others cited the time commitment, desire to begin hormone treatment immediately, discomfort with the concept of placebo treatment, inadequate financial compensation, and general anxiety about participating in a study.

Based on psychiatric screening, participants with a current Axis 1 DSM-IV diagnosis, current suicidal ideation, past history of suicide attempt, and/or a Beck Depression Inventory score greater than 15 were eliminated from further participation in the study and referred for treatment if necessary (N=23). A further 18 women who did not meet criteria for menopause (values of luteneizing hormone (LH) and follicle stimulating hormone (FSH) > 20 IU/L) were eliminated. Eight women withdrew from further participation because they had changed their minds, and 3 were advised against participation by their physician (see description of medical examination below).

Therefore, 72 women in total met all medical, psychiatric and hormonal entrance criteria and were randomly assigned to one of two treatment groups: 36 participants were

assigned to treatment with exogenous estrogen for 12 weeks and 36 participants received a placebo for the same period of time. Groups were stratified based on past history of affective disturbance. A past history of affective disturbance was defined as one or more major depressive episodes, severe premenstrual dysphoria or postpartum depression as assessed by the SCID for DSM-IV and a reproductive history questionnaire (see materials).

On the day of the psychiatric evaluation and prior to any testing, all participants gave informed, signed consent by means of a form approved by a university hospital ethics committee (see Appendix 2) and were reassured that they could withdraw from the study at any time. Financial compensation was provided for lost work time and transportation costs in the following manner: (1) initial interview: $30, (2) study day 1: $40, (3) study day 2: $50.

Materials:

I - Test Battery:

a) Structured Clinical Interview for DSM-IV (SCID) (First, Williams, Gibbon, & Spitzer, 1997):

The SCID consists of a semi-structured interview designed to diagnose psychopathology using the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (APA, 1994). The length of the interview is influenced by the extent of psychopathology present, but it typically takes between1½ and 2 ½ hours.

b) Reproductive & Medical History:

This 22-item questionnaire (devised specifically for this study) is designed to assess reproductive history, including oral contraceptive use, menstrual cycle characteristics, premenstrual mood changes, number of pregnancies, pregnancy complications and postpartum affective disturbance and characteristics of the menopause (including date of last menstrual period). There is also a short section on personal and family medical and psychiatric history (Appendix 3).

c) Menopausal Index (Blatt, Wiesbader, & Kupperman, 1953) (revised by Sherwin, 1983):

This 27-item paper-and-pencil questionnaire was administered prior to and following hormone treatment to monitor the frequency and severity of menopausal symptoms on interval rating scales ranging from 0 (never) to 7 (very often) (Appendix 4).

d) Family Instrument for Genetic Studies (FIGS) (Nurnberger et al., 1994):

This semi-structured interview is designed to elicit information regarding family history of psychopathology. It can take up to 1 hour to complete.

e) Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961):

The BDI is a 21-item, individually administered multiple choice, self-report instrument designed for screening and quantifying the severity of depressive affect. It generally takes 5-10 minutes to complete. Participants were instructed to assess their current mood, rather than their mood during the past week. Studies of the internal consistency and stability of the instrument indicate a high degree of reliability (Beck & Steer, 1987). Concurrent discriminative and construct validity have also been demonstrated for the BDI (Gallagher, 1986). There is a large body of evidence supporting the conclusion that the BDI has adequate reliability and validity for clinical and research purposes in general adult samples of varying ages (Gallagher, 1986).

f) Profile of Mood States - Bipolar Form (POMS-BI) (Lorr & McNair, 1982):

The POMS-BI is a 72-item, self-report affective inventory. It provides measures of 6 bipolar mood states each rated on a 4 point scale, where 0=much unlike this and 3=much like this. The dimensions include: composed-anxious, agreeable-hostile, elated-depressed, confident-unsure, energetic-tired, and clearheaded-confused. It typically takes 5 minutes to complete.

g) Visual Analogue Mood Scale (VAMS) (Bond & Lader, 1974):

The VAMS consists of sixteen 100-mm horizontal lines, each representing a bipolar dimension of mood state, on which the participant is instructed to place a perpendicular mark that best describes their mood state.

II- Drugs:

The active estrogen treatment consisted of oral conjugated equine estrogen 0.625 mg/day (Premarin, Wyeth-Ayerst Laboratories, Montreal) for twelve weeks. This is the standard dose for treatment of postmenopausal women. The placebo treatment consisted of sugar pills that were visually indistinguishable from the active hormonal tablets, administered once a day for twelve weeks.

Random assignment to Premarin 0.625 mg/day or to placebo was under the control of the Chief Pharmacist at the Allan Memorial Institute of the Royal Victoria Hospital, Montreal, Canada. Two random assignment lists were generated, one for women with a past history of affective disturbance and one for women with no history of affective disturbance. Drugs were dispensed by the pharmacists at the Allan Memorial Institute of the Royal Victoria Hospital, and the experimenter and the participants were blind to the treatment.

III - Amino Acid Mixtures:

The amino acid mixtures were prepared following the protocol of Young and associates (1985), and modified for use with female participants by decreasing the total amount of amino acids in the T- drink from 100 g to 85.8 g to adjust for sex differences in body weight (Ellenbogen et al., 1996). The tryptophan deficient (T-) mixture consisted of 15 amino acids weighing 85.8 g which included: L-alanine 4.6 g, L-arginine 4.1 g, L-cysteine 2.3 g, glycine 2.7 g, L-histidine 2.7 g, L-isoleucine 6.7 g, L-lysine monohydrochloride 9.2 L-methionine 2.5 g, L-phenylalanine 4.8 g, L-proline 10.2 g, L-serine 5.8 g, L-threonine 5.4 g, L-tyrosine 5.8 g, and L-valine 7.4 g. The balanced amino acid mixture (B) contained the same amino acids plus 1.9-g L-tryptophan. Because of the unpleasant taste of methionine, cysteine and arginine, these amino acids were encapsulated and administered separately. The amino acid mixture was prepared within a few minutes of oral administration by mixing the powdered amino acids with 135 ml water, 45 ml chocolate syrup and 0.6 g of sodium cyclamate (an artificial sweetener). For

participants who dislike chocolate, an alternate mixture was available which consisted of the powdered amino acids, 180 ml of orange juice from concentrate, and 1.1 g of sodium cyclamate.

IV- Hormone and Tryptophan Assays:

The free (non-albumin bound) plasma tryptophan concentration was assessed by determining the concentration of tryptophan found in an ultrafiltrate of plasma prepared at 25° C under an atmosphere containing 5% carbon dioxide in a centrifugal ultrafilter (MPS-1, Amicon Inc., Beverly, Mass.) through YMT membranes (Millipore Waters, Bedford, Mass.). Tryptophan in the ultrafiltrate, and in plasma, was measured by high performance liquid chromatography on a reverse-phase column (μBondapak C_{18}, Millipore Waters) with fluorometric detection.

Blood samples for the steroid hormones were centrifuged and the plasma stored at -20°C until analysis. Estradiol and estrone levels were determined in duplicate by radioimmunoassay with the use of antisera after extraction into ethyl ether. Charcoal was used as an absorbent.

Procedure:

I - Design Overview:

A prospective, double-blind, placebo-controlled, cross-over design was employed. For participants who met all inclusion criteria (hormonal, medical and psychiatric) assignment to treatment group (estrogen or placebo) was random and double-blind. Participants were also stratified based on previous history of depression. All participants completed one Acute Tryptophan Depletion (T-) test session and one nutritionally balanced amino acid (B) control session, scheduled at least one week apart, during the final 2 weeks of the 12-week treatment phase. Test sessions were counterbalanced for order and both participants and experimenter were blind to the nature of the amino acid mixture used during a given test session. On the day prior to each test session, participants were provided with pre-packaged, pre-cooked, low-protein meals or were instructed on how to prepare them themselves when that was more convenient for the participant (e.g. if they lived at a considerable distance from the testing site) (see Appendix 5). These meals, required to be eaten for breakfast, lunch and dinner on the day before the T- and B test sessions, provided 160 mg tryptophan/24 h, 22.6 g protein/24 h, and 2212 kcal/24 h and were identical to those used in previous studies (Benkelfat et al., 1994; Ellenbogen et al., 1996). A schematic diagram of the experimental design appears in Figure 2 at the end of the section.

II- Psychiatric Evaluation:

Following the telephone screen, women who agreed to schedule an appointment for a psychiatric interview (N=126) were instructed to begin an overnight fast on midnight of the night prior to their scheduled appointment. Prior to the initial interview, 20 ml of blood was obtained via venipuncture by a registered nurse or blood technician to determine levels of LH, FSH, estradiol, estrone, and free (non-albumin bound) and total tryptophan.

The initial interview and baseline evaluation included the Structured Clinical Interview for DSM-IV (non-patient version), the BDI and the Reproductive and Medical History Questionnaire. Family history of psychiatric disturbance was evaluated using the Family interview for Genetic Studies. Baseline mood state was also evaluated, for comparison purposes, using the measures that were employed during the testing phase. These included the POMS-BI and the Menopausal Index.

In total, the baseline test session generally took approximately 3-4 hours but, in some cases, it took as long as 5-6 hours depending on the amount of psychopathology reported by the participant.

Based on these results, participants who were deemed suitable for participation in the study (N=75) were provided with a letter for their gynecologist briefly explaining the nature of the study (see Appendix 6) and a form for their gynecologist to sign indicating

that the participant had no contraindications to participating in the study. Medical exclusion criteria included an estrogen-dependent cancer, breast cancer in a first-degree relative or uterine bleeding of unknown origin. The medical examiner confirmed that potential participants did not have any serious acute or chronic illnesses (e.g. diabetes or coronary heart disease (see Appendix 7). If the participant had been examined by her own gynecologist within the previous six months, the written confirmation from him/her that no contraindications to estrogen therapy existed for the participant was obtained. Women who did not have their own gynecologist were provided with a referral. Three participants were denied permission by their physicians to participate, leaving the total number of women entering the treatment phase of the study at N=72. During the 12-week course of hormone treatment and prior to the Acute Tryptophan Depletion (ATD) test days, eight women withdrew (four in the placebo group and four in the estrogen group - see Results section for details) leaving a total number of 64 participants. Sixty-four women entered the ATD testing phase of the study, and three withdrew (two estrogen and one placebo) between the first and second test day (two following the T- day and one following the B day), leaving a total of 61 subjects who completed the entire protocol.

III - Tryptophan Depletion Test Sessions:

Following eleven weeks of treatment with either estrogen or placebo, all participants were scheduled for two test sessions (one week apart). On each of the test days, participants ingested one of the two amino acid mixtures (either T- or B) which were presented in a double-blind, counterbalanced fashion.

On the day prior to the test sessions, participants consumed only the low-protein diet provided for them and they began an overnight fast at midnight. Appointments were scheduled between 9-10 a.m. the next morning, at which time mood state was evaluated (using the POMS-BI, the VAMS, the BDI and the menopausal index) and 20 ml of blood was obtained via venipuncture to determine plasma levels of estradiol, estrone, and free and total tryptophan. Participants were then provided with either a mixture of amino acids devoid of tryptophan (T-) or a nutritionally balanced (B) control mixture. For the next five hours, participants remained alone in a comfortable room specifically designated for ATD studies. Affectively neutral videotapes and reading material were available. Participants were asked not to sleep, and were monitored frequently. Another sample of blood was obtained five hours following ingestion of the drink and mood state was then re-evaluated. Following testing, participants were given a tryptophan supplement and a high-protein snack to restore their tryptophan levels. The tryptophan supplement that was used is available by prescription in Canada and has not been associated with any cases of eosinophilia myalgia syndrome. Participants were supervised for up to one hour following the test session, and phone contact was maintained for up to 24 hours, if necessary. If participants had any questions or concerns, they were encouraged to contact the investigators.

Data Analysis:

Differences between the two groups with respect to demographic and baseline variables were analyzed with two-tailed, independent samples t-tests (with equal variances assumed). All 61 subjects were used in these analyses except where indicated.

Changes in estrone and estradiol values, hot flushes, cold sweats, and mood over the course of hormone treatment (from pre-treatment to 11 weeks post-treatment) were evaluated using two-way mixed ANOVAs. Hormone Status (estrogen vs. placebo) was the between group factor, and Day (screening vs. Test day-1 (11 weeks post-treatment)) was the within-subject, repeated measure.

ATD data were converted to change scores to control for intra-subject variability, and was analyzed using a two-way mixed ANOVA. Hormone Status (estrogen vs. placebo) was the between group factor, and Drink (T- vs. B) was the within-subject, repeated measure. Planned comparisons were performed for statistically significant factor effects or factor interactions.

Power Analysis:

Controlling for Type I error at an alpha level of .05 and power at .80, a sample size of 36 per group allowed for the detection of a difference of 0.35 standard deviations between standardized means (Cohen, 1988).

Ethical Issues:

I-Estrogen Replacement Therapy:

While there are several indications for estrogen replacement therapy in postmenopausal women (e.g. treatment of hot flushes, atrophy of tissues of the reproductive tract, prevention of osteoporosis and protection against heart disease in at-risk populations), ERT is not universally prescribed and often depends on the preference of the individual patient. Assignment to the placebo group for 12 weeks, then, did not constitute a failure to provide suitable treatment for this short period of time.

For menopausal woman with an intact uterus, a progestin is usually added to the estrogen replacement regimen in order to protect against endometrial hyperplasia that could occur with treatment with estrogen alone. However, the administration of estrogen-alone for 3 months does not cause endometrial hyperplasia and quarterly administration of a progestin may, in fact, be preferable to monthly administration (Ettinger et al., 1994). Following completion of the study, medroxyprogesterone acetate (Provera) was added to the regimen of women who had been in the estrogen group and who elected to continue with hormone replacement therapy (prescribed by their individual physicians). The women in the placebo group were offered active treatment when the double-blind code was broken at the conclusion of the study.

II-Acute Tryptophan Depletion:

As mentioned above, ATD is a technique which is short-lived, non-toxic, and makes use of normal metabolic processes to experimentally alter brain function. The amino acids are mixed in the same proportions as they occur in human milk and are therefore suitable for use with human participants (Young, Ervin, Pihl, & Finn, 1989). However, some short-term negative consequences of ingestion of the amino acids sometimes occur. These include: (1) feelings of nausea or "bloating", which usually subside in one to three hours, (2) lowered or heightened energy levels (3) drowsiness or tiredness, or heightened alertness, and (4) a possible change in mood. When they occurred, these effects were short-lasting and were usually completely reversed following the balanced meal and tryptophan supplement given at the end of each study day. Participants who continued to have symptoms at the end of the day remained in the laboratory for a short time under medical observation. They were then driven home by taxi if required (N=2). They received a follow-up telephone call that evening and on the subsequent day to ensure that the symptoms(s) had remitted.

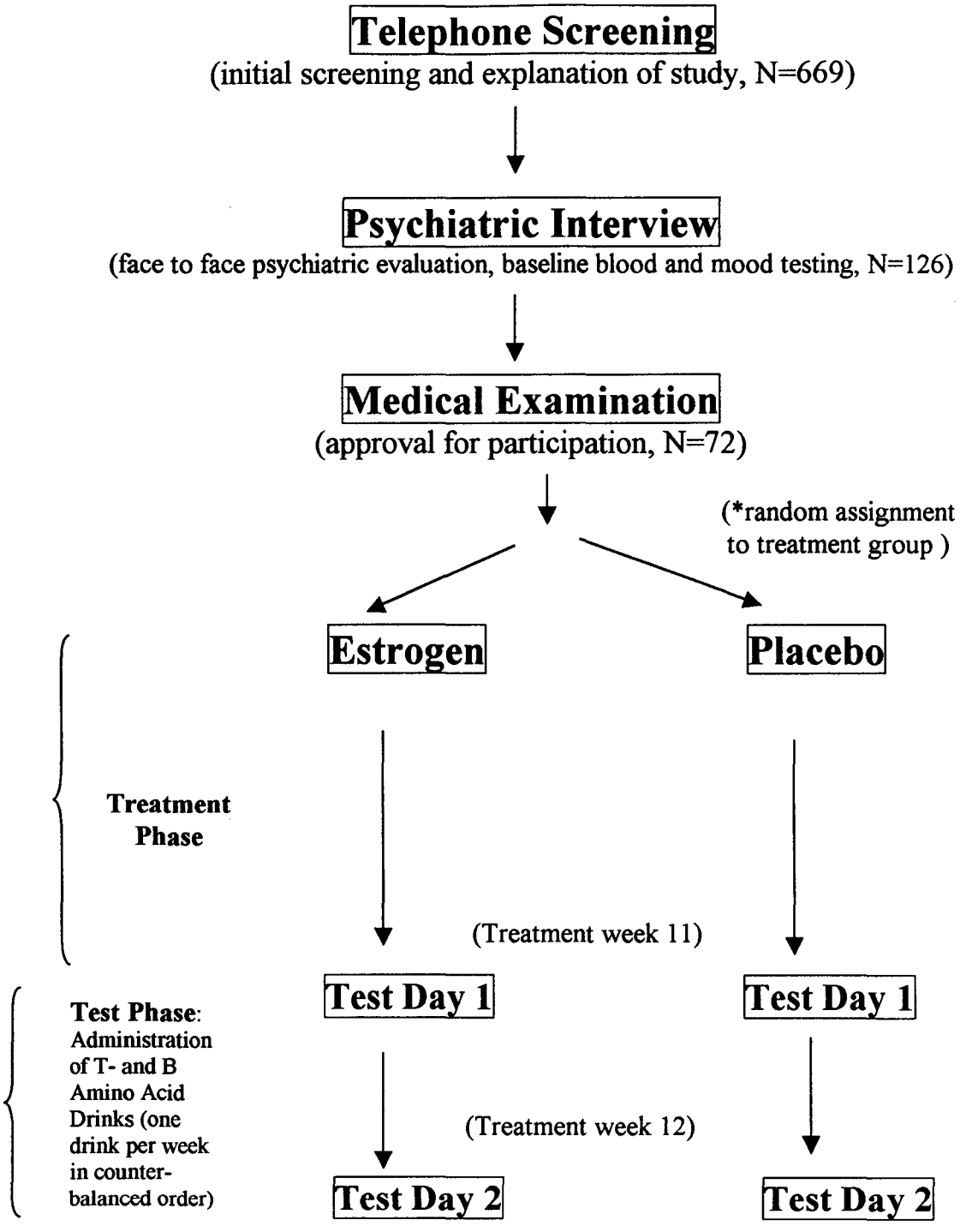


Figure 2: Experimental Design

RESULTS

Seventy-two women entered the hormone treatment phase of the study. During the 12-week course of hormone treatment and prior to the ATD test days, eight women withdrew (four in the placebo group and four in the estrogen group) leaving a total number of 64 participants. Two women withdrew for medical reasons (arm surgery, placebo group; unusual mammogram result, estrogen group). Four women cited side effects of the hormone or placebo treatment as their reason for withdrawing (breast sensitivity, vaginal discharge) (three in the estrogen group, one in the placebo group), one participant found full time employment and was therefore unable to commit the time (placebo group), and one woman reported anxiety regarding the testing situation (estrogen group).

Sixty-four women entered the ATD testing phase of the study, and three withdrew (two estrogen and one placebo) between the first and second test day (two following the T- day and one following the B day), leaving a total of 61 subjects who completed the entire protocol. Three of the 61 women were excluded from all data analysis. Two of these women had been in the estrogen group and had unusual hormone profiles (e.g. extreme discrepancies between the two test days that occurred one week apart which suggested erratic compliance with hormone treatment). The data from one placebo-treated woman was eliminated because her mood questionnaire data displayed patterns typical of a major depressive disorder with prominent morning symptoms, suggesting that she had become clinically depressed.

Therefore, the total number of participants in the hormone treatment phase analysis was 61 and the total number of participants in the ATD test phase was 58 (61 women who completed the total protocol minus the three women excluded from the analyses).

1) Personal and Demographic Data:

a) Age:

The ages of the 61 participants in the study ranged from 44.9 to 63 years with a mean of 52.88 years (+/- .49). Age did not differ between the Estrogen and Placebo groups (t (59)=.327, p=.745) (Table 1).

b) Weight:

Weight data were not available for four participants, so mean weight was calculated based on data from the remaining 57 women. Mean weight of these participants was 153.88 (+/-3.9) pounds and did not differ between groups (t (55)=-.284, p=.778) (Table 1).

c) Menopausal History:

Of the 61 participants, 50 (82%) had undergone spontaneous menopause (i.e. non-surgical), 2 (3%) had had hysterectomies with both ovaries left intact and therefore also

underwent natural menopause, 4 (7%) had had hysterectomy with unilateral oophorectomy, and 5 (8%) had had hysterectomy with bilateral oophorectomy, thus rendering them surgically menopausal (2 women from the estrogen group and 3 women from the placebo group).

Age at menopause and duration of menopause (as defined as months since last menstruation) were ascertained based on participant's retrospective reports of their last menstrual cycle. Women who had undergone hysterectomy-only or hysterectomy with unilateral oophorectomy were excluded from this analysis. In total, 55 women were included in this analysis. The average age of menopause for all women was 49.4 (+/-.51) years, with a range of 39.7 to 56 years. The two treatment groups did not differ with respect to age at menopause ($t(53)=1.008$, $p=.318$). Surgically menopausal women ($n=5$) had significantly earlier onset of menopause than naturally menopausal women (mean age=45.5 (+/-.1.93)) ($t(53)=-2.517$, $p=.015$). The average age of menopause excluding those who had undergone surgical menopause was 49.78 (+/-..50) years.

Mean duration of menopause (as defined by number of months since last menstruation) was 43.8 months (+/- 6.3), while median number of months since last menstruation was 24 months. There were no significant differences between treatment groups with respect to number of months since last menstruation ($t(53)=-0.51$, $p=.959$) (Table 1).

Table 1: Personal Characteristics & Menopausal History				
	Age (years)	**Age at Menopause (years)**	**Months since Menstruation**	**Weight (lbs.)**
Entire Sample	52.9 (+/-.49) (n=61)	49.4 (+/-.51) (n=55)	43.8 (+/-.6.3) (n=55)	153.88 (+/-3.9) (n=61)
Estrogen Group	53.0 (+/-.55) (n=36)	49.8 (+/-.68) (n=31)	43.6 (+/-9.3) (n=31)	153.00 (+/-5.2) (n=36)
Placebo Group	52.7 (+/-.89) (n=25)	48.8 (+/-.77) (n=24)	44.2 (+/-8.3) (n=24)	155.22 (+/.5.7) (n=25)

d) Level of Education:

Overall, the median classification of education level was a 2-year college degree and did not differ between groups ($x^2 = 4.958$, df=6; p = .549). However, these degrees were largely secretarial business school and hospital-based nurses' training that were popular 30 years ago and may be below the educational requirements for similar job classifications today. All levels of education were represented with the exception of individuals in the lowest classification (<7th grade), who were excluded due to protocol requirements. Most of the women who participated had graduated from high school (93%), and a relatively large proportion had graduate level degrees (16%) (Table 2). In general, these figures are consistent with those of the general population of Canada, where the median years of education achieved is 12.7 years (Statistics Canada, 1996 Census – National Tables).

Table 2: Education Level of Participants								
	< 7th grade	Part High School	High School Degree	Part college	2 year college degree	4 year college degree	Part grad. School	Grad. degree
Entire Sample (n=61)	0% (0)	7% (4)	21% (13)	18% (11)	13% (8)	23% (14)	2% (1)	16% (10)
Estrogen Group (n=36)	0% (0)	8% (3)	17% (6)	19% (7)	11% (4)	22% (8)	0% (0)	22% (8)
Placebo Group (n=25)	0% (0)	4% (1)	28% (7)	16% (4)	16% (4)	24% (6)	4% (1)	8% (2)

e) Marital Status:

The majority of participants in this study were married (59%). Twenty-eight percent of women were divorced and 11% were separated, widowed or never married (Table 3). Overall, the estrogen group and the placebo group did not differ in terms of marital status ($x^2 = 2.057$, df=4; p = .725). However, a somewhat larger proportion of participants were married compared to the norms for women in Canada (48%) (Statistics Canada, 1996). This may reflect the limited age-range of the participants in this study, which eliminated the youngest segments of the population who may be less likely to be married.

Table 3: Marital Status of Participants					
	Married	**Divorced**	**Separated**	**Widowed**	**Never Married**
Entire Sample (n=61)	59% (36)	28% (17)	3% (2)	3% (2)	7% (4)
Estrogen Group (n=36)	56% (20)	28% (10)	6% (2)	3% (1)	8% (3)
Placebo Group (n=25)	64% (16)	28% (7)	0% (0)	4% (1)	4% (1)

2) Hormone Levels:

a) Pretreatment baseline FSH and LH values:

At pretreatment baseline, the Estrogen group had a mean FSH value of 78.86 (+/-4.21) IU/L and the Placebo group had a mean FSH value of 81.02 (+/-7.3) IU/L (table 4). There were no significant differences between the two groups on this measure (t(59)-.273, p=.786). The normal range for FSH values in postmenopausal women is 25-88 IU/L, while the normal range for pre-menopausal women is 0.9-7.6 IU/L during the follicular phase of the menstrual cycle and 3.7-25 IU/L at mid-cycle.

Mean values of LH at baseline evaluation were 48.65 (+/-2.32) for the Estrogen group and 50.85 (+/-4.95) for the Placebo group. There were no significant differences between the two groups (t(59)=-.443, p=.659) (table 4). The normal range for LH values in postmenopausal women is 30-72 IU/L, while the normal range for pre-menopausal women is 0.6-11 IU/L during the follicular phase of the menstrual cycle and 17-49 IU/L at mid-cycle. Thus, all participants had FSH and LH values within the postmenopausal range at pretreatment baseline.

b) Pretreatment Baseline Estrone and Estradiol Values:

At pretreatment baseline, the two groups did not differ significantly with respect to plasma estradiol ($t(59)=-.171$, $p=.865$) or estrone values ($t(59)=-.709$, $p=.481$). The mean estradiol values at baseline were 111.76 (+/- 17.97) pmol/L for the Estrogen group and 116.32 (+/- 18.88) pmol/L for the Placebo group (table 4). Based on the norms provided by the lab that carried out the assays, the normal range of estradiol values for postmenopausal women is 0-110 pmol/L, while the normal range for cycling women is 147-441 pmol/L during the follicular phase and 447-1101 pmol/L during the luteal phase. Therefore, the mean estradiol values of the women in this sample were at the upper limit of the postmenopausal range.

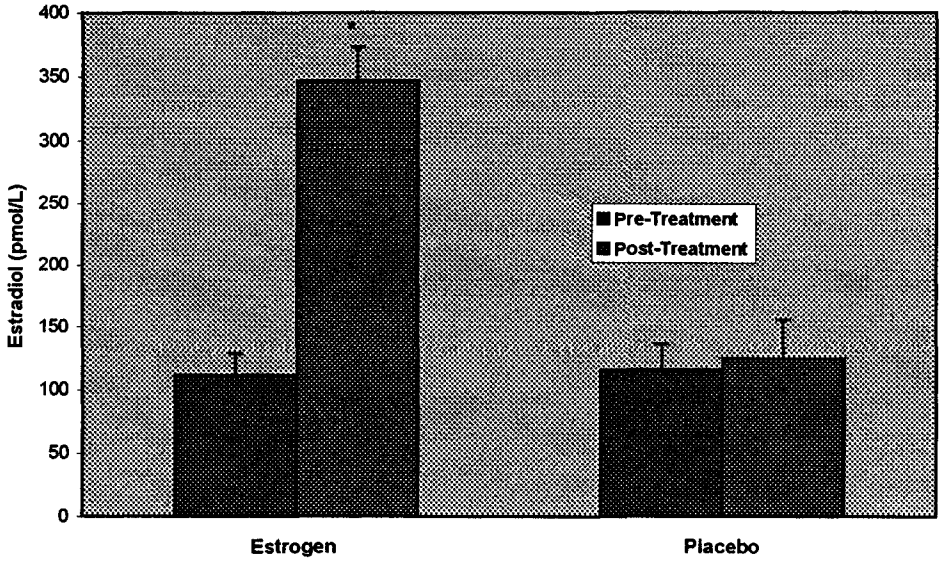
The mean values of estrone at baseline were 86.43 (+/- 9.74) pmol/L for the Estrogen group and 99.29 (+/- 16.66) pmol/L for the Placebo group (table 4). The normal range of estrone for postmenopausal women is 0-148 pmol/L, while the normal range for cycling women is 111-370 pmol/L during the follicular phase, and 180-420 pmol/L during the luteal phase. Therefore, the mean values of the women in this sample were within the normal range for postmenopausal women.

Table 4: Pretreatment Baseline Hormone Levels				
	FSH (IU/L)	**LH** (IU/L)	**Estradiol** (pmol/L)	**Estrone** (pmol/L)
Estrogen Group (n=36)	78.86 (+/- 4.2)	48.65 (+/- 2.3)	111.76 (+/-18)	86.43 (+/- 9.74)
Placebo Group (n=25)	81.02 (+/-7.3)	50.85 (+/- 4.95)	116.32 (+/-19)	99.29 (+/- 16.7)
Postmenopausal Norms	25-88	30-72	0-110	0-148

c) Effects of 12-weeks of Treatment with Estrogen or Placebo:

Treatment with exogenous estrogen for 12 weeks caused a significant increase in plasma estradiol values compared to both pretreatment baseline and to placebo treatment for the same time period (figure 3). This was demonstrated by results of a two-way mixed ANOVA (with Day [Pretreatment vs. Post-treatment] as the within subjects factor and Hormone Status as the between groups factor) which revealed significant main effects of Day ($F(1,59)=23.687$, $p=.000$) and Hormone Status ($F(1,59)=21.515$, $p=.000$) and a significant interaction of Day X Hormone Status ($F(1,59)=20.559$, $p=.000$). Estradiol values increased from 111.8 (+/-17.1) pmol/L at pretreatment baseline to 346.8 (+/-25.9) pmol/L following 12 weeks of treatment in the group that received exogenous estrogen. Estradiol values in the Placebo group did not change appreciably from baseline (116.3 (+/-20.5) pmol/L) to post-treatment 12 weeks later (124.6 (+/- 31.1) pmol/L).

Figure 3: Effect of Treatment with Exogenous Estrogen or Placebo on Plasma Estradiol Values

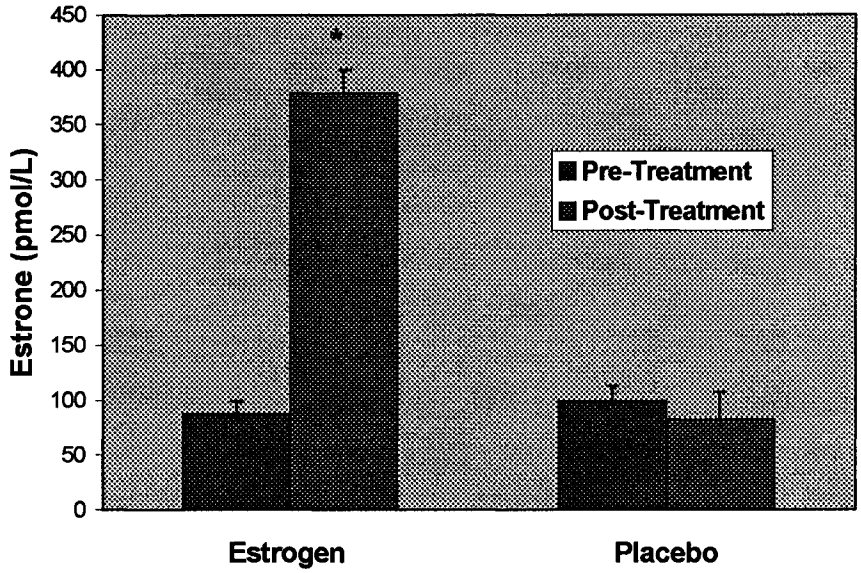


Treatment with exogenous estrogen (premarin .625 mg/day for 12 weeks) (n=36) results in significantly increased plasma estradiol values as compared to placebo treatment (n=25) for the same period of time. Repeated measures ANOVA reveals significant main effects of day (p=.000) and treatment (p=.000) and a significant interaction of day X treatment (p=.000).

Treatment with exogenous estrogen also resulted in significant increases in plasma estrone values compared both to pretreatment baseline and to placebo treatment for the same time period (figure 4). This was demonstrated by a two-way mixed ANOVA which revealed significant main effects of Day ($F(1,59)=61.607$, $p=.000$) and Hormone Status ($F(1,59)=50.189$, $p=.000$) and a significant interaction of Day X Hormone Status ($F(1,59)=77.541$, $p=.000$).

Estrone values increased from 86.4 (+/-11.6) pmol/L at pretreatment baseline to 369.1 (+/-21.9) pmol/L following 12 weeks of treatment in the group receiving exogenous estrogen. Estrone values in the Placebo group did not change significantly from pretreatment baseline (99.3 (+/-13.9) pmol/L) to post-treatment 12 weeks later (82.61(+/-26.2) pmol/L).

Figure 4: Effect of Treatment with Exogenous Estrogen or Placebo on Plasma Estrone Values



Treatment with exogenous estrogen (premarin .625 mg for 12 weeks)(n=36) results in significantly increased plasma estrone values as compared to placebo treatment (n=25) for the same time period. Repeated measures ANOVA reveals significant main effects of day (p=.000) and treatment (p=.000) and a significant interaction of day X treatment (p=.000).

2) Menopausal Symptoms:

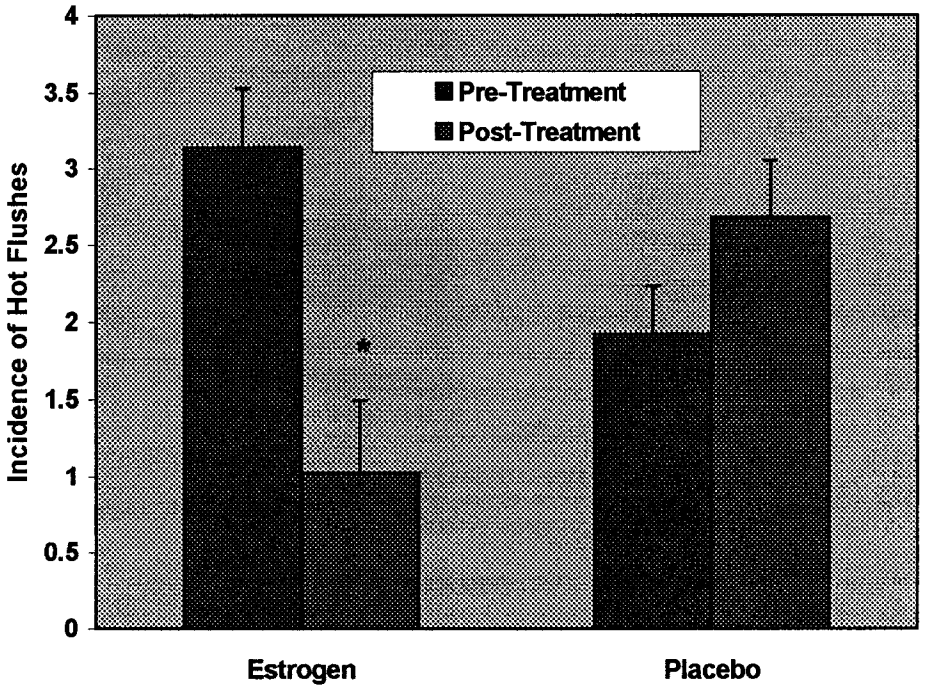
a) Hot flushes:

At pretreatment baseline, the women in the estrogen group were experiencing hot flushes to a greater degree than those in the placebo group. On a scale of 0 to 7, where 0=almost never and 7=very often, the mean baseline score of women in the Estrogen group was 3.14 (+/-.39) while the mean score for the Placebo group was significantly lower (1.92 (+/-.47) ($t(59)=2.01$, $p=.049$). For this reason, analysis of the impact of estrogen treatment on the incidence of hot flushes was done in two ways. First the raw scores were analyzed using a 2-way mixed ANOVA (with Day as the within-subject, repeated measure and Hormone Status as the between group measure). Second, change scores (day 1 of testing [after 11 weeks of treatment with estrogen or placebo] subtracted from hormone pretreatment scores) were analyzed using an independent samples t-test in order to control for the impact of the pretreatment baseline difference.

In both analyses, treatment with estrogen for 12 weeks significantly reduced the incidence of hot flushes while treatment with placebo did not (figure 5). A two-way mixed ANOVA on the raw scores revealed a significant main effect of Day ($F(1,59)=4.565$, $p=.037$) and a significant interaction of Hormone Status X Day ($F(1,59)=20.613$, $p=.000$). This indicates that treatment with estrogen was associated with a significant reduction in hot flushes from pretreatment baseline (3.14 +/-.39) to

post-treatment (1.03 +/-.31). Treatment with placebo, however, did not cause a pre- (1.92 +/-.47) to post-treatment (2.68 +/-.37) change in hot flush frequency. An independent samples t-test on change scores also revealed that the estrogen group had significantly greater improvement in hot flushes (2.11 (+/- .38)) as compared to the placebo group (-.76 (+/-.52)) (df=59, t=4.54, p=.000).

Figure 5: Treatment with Exogenous Estrogen Reduces Hot flushes as Compared to Placebo



Bars represent scores on the item "frequency of hot flushes" on the Menopausal Index. Repeated measures ANOVA reveals a significant main effect of day (p=.037) and a signficant interaction of treatment by day (p=.000). Treatment with exogenous estrogen for 12 weeks (n=36) significantly reduces the incidence of hot flushes, while treatment with placebo does not.

b) Cold Sweats

At pretreatment baseline, the two groups were equivalent with respect to the extent to which they reported "cold sweats" as measured by the Menopausal Index (estrogen group mean =.67 (+/-.22), placebo group mean = .76 (+/-.23) (t(59)= -.289, p=.77).

In order to assess the effect of treatment with exogenous estrogen on reports of "cold sweats", a two-way mixed ANOVA (with Drink as the within group factors and Hormone Status as the between group factor) was performed. There were no significant effect of day (pre vs. post) (F(1, 59)=.004, p=.948) and no significant interactions of Day X Status (F(1,59) = 2.901, p=.094). However, the changes were in the expected directions in that cold sweat frequency tended to decrease following treatment with estrogen and tended to increase following treatment with placebo (table 5).

Table 5: Cold Sweats Pre and Post 12 weeks of Treatment with Estrogen or Placebo

	Cold Sweats Pre	**Cold Sweats Post**
Estrogen Group (n=36)	.67 (+/- .22)	.28 (+/- .14)
Placebo Group *(n=25)*	.76 (+/- .23)	1.12 (+/- .4)

c) Total Menopausal Index Scores

At pretreatment baseline, there were no significant differences in overall Menopausal Index scores between the two treatment groups (estrogen group mean = 40.72 (+/- 3.65), placebo group mean = 39.68 (+/- 5.65) (t(59)= .162, p=.87), suggesting that they were equally symptomatic.

In order to assess the effect of treatment with exogenous estrogen on overall Menopausal Index scores, a two-way mixed ANOVA (with Drink as the within group factors and Hormone Status as the between group factor) was performed. A significant effect of day (pre vs. post) (F(1, 59)=18.45, p=.000) but no significant interaction of Day X Status (F(1,59) = .938, p=.34) occurred, indicating that symptoms were reduced for all participants irrespective of treatment status (table 6).

Table 6: Total Menopausal Index Pre and Post 12 weeks of Treatment with Estrogen or Placebo

	Menopausal Index Pre [a]	**Menopausal Index Post** [b]
Estrogen Group (n=36)	40.72 (+/- 3.65)	27.31 (+/- 3.25)
Placebo Group (n=25)	39.68 (+/- 5.65)	31.2 (+/- 4.06)

a significantly > *b*, p < .001

3) Tryptophan Levels:

a) Pretreatment Baseline Tryptophan Levels:

On the morning of pre-treatment baseline (prior to any experimental intervention) there were no significant differences between the two groups in terms of total plasma tryptophan (estrogen group mean = 10.04 (+/-.19) μg/ml, placebo group mean = 9.7 (+/-.20) μg/ml) ($t(57)=1.231$, $p=.223$)) or free plasma tryptophan (estrogen group mean = 1.48 (+/-.004) μg/ml, placebo group mean = 1.58 (+/-.007) μg/ml) ($t(55)=-1.249$, $p=.217$)) (table 7).

Table 7: Pretreatment baseline levels of Total and Free Plasma Tryptophan (μg/ml)

	Total	Free
Estrogen Group (n=36)	10.04 (+/- .19)	1.48 (+/- .004)
Placebo Group (n=25)	9.7 (+/- .2)	1.58 (+/- .007)

b) Post Hormone Treatment ATD Test Day Baseline Values:

On ATD test days, prior to the participants' ingestion of the amino acid drinks, there were no significant differences between the two groups in terms of total or free plasma tryptophan (T- day total plasma tryptophan: ($t(56)=1.382$, $p=.173$), T- day free plasma tryptophan: ($t(55)=-.520$, $p=.605$), B day total plasma tryptophan: ($t(56)=.763$, $p=.448$), B day free plasma tryptophan: ($t(56)=.121$, $p=.904$)) (table 8).

Table 8: ATD Test day baseline levels of Total and Free Plasma Tryptophan (μg/ml)				
	T- Day Total	**B Day Total**	**T- Day Free**	**B Day Free**
EstrogenGroup (n=36)	9.76 (+/- .19)	9.91 (+/- .2)	1.56 (+/- .004)	1.64 (+/- .004)
Placebo Group (n=25)	9.33 (+/- .25)	9.68 (+/- .2)	1.61 (+/- .008)	1.63 (+/- .008)

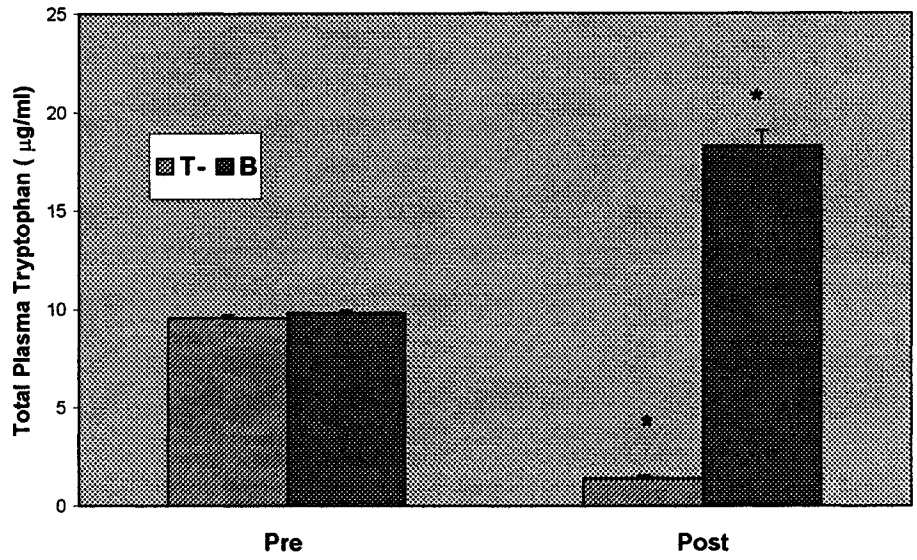
c) Effect of Amino Acid Drinks on Free and Total Tryptophan Levels:

Ingestion of the tryptophan deficient amino drink (T-) resulted in a significant reduction in total plasma tryptophan levels in both treatment groups, while ingestion of the balanced amino acid drink significantly increased total plasma tryptophan levels (figure 6). A two-way mixed ANOVA (with Drink and Test Time as the within group factors and Hormone Status as the between group factor) revealed a significant main effect of Drink ($F(1, 56)=457.603$), $p<.001$) as well as an interaction of Drink X Test Time ($F(1,56)=453.901$, $p<.001$). The T- drink lowered total plasma tryptophan levels by 86% (from 9.58 (+/- .15) μg/ml in the morning to 1.38 (+/- .13) μg/ml 5 hours following the drink; $t(58)=38.852$, $p<.001$). The B drink increased levels by 187% (from 9.81 μg/ml (+/.14) in the morning to 18.36 (+/- .74) μg/ml five hours following the drink) ($t(58)=-11.859$, $p=.001$). There were no significant between group differences.

A similar pattern was found with respect to free plasma tryptophan (figure 7). Two-way mixed ANOVA revealed a significant main effect of Drink ($F(1,55)=306.023$, $p<.001$) as well as an interaction of Drink X Test Time ($F(1,55)=343.757$, $p<.001$) on

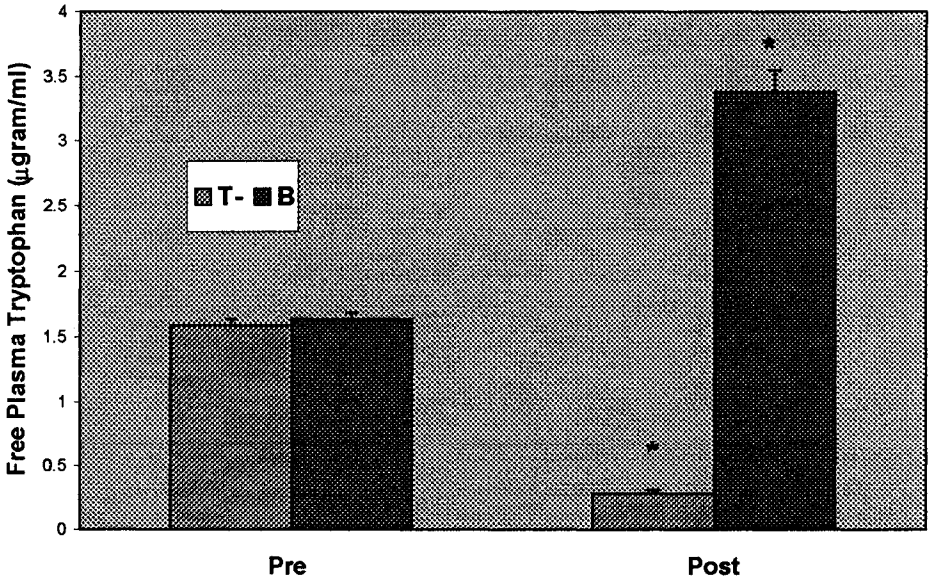
free plasma tryptophan levels. The T- drink lowered free plasma tryptophan levels by 82% (from 1.58 (+/- .05) μg/ml in the morning to .2712 (+/-.03) μg/ml five hours following the drink; t (56)=22.636, p=.000) while the B drink increased levels by 209% (from 1.64 (+/- .04) μg/ml to 3.43 (+/- .16) μg/ml five hours following the drink (t (57)=-11.358, p<.000). There were no significant between group differences.

Figure 6: Effect of Tryptophan Deficient and Balanced Amino Acid Drinks on Total Plasma Tryptophan



Blood was taken from participants at 9AM on 2 test days (Tryptophan deficient, B=Balanced Amino Acid drink) prior to ingesting the drink and again 5 hours later. Repeated Measures ANOVA (n=58) revealed a significant main effect of test day (p=.000) as well as an interaction between day and time (p=.000). The T- drink lowered total plasma tryptophan by 86% (p<.001) while the B drink increased levels by 187% (p<.001).

Figure 7: Effect of Tryptophan Deficient and Balanced Amino Acid Drinks on Free (non-albumin bound) Plasma Tryptophan



Blood was taken from participant at 9AM on both test days (T-, B) prior to ingesting the amino acid drink and again 5 hours later. Free plasma tryptophan was taken as the concentration of tryptophan in an ultrafiltrate of plasma. Repeated Measures ANOVA (n=58) revealed a significant main effect of test day (p=.000) as well as an interaction between day and time (p=.000). The T- drink lowered free plasma tryptophan by 82% (p<.001) while the B drink increased levels by 206% (p<.001).

4) Relationship between Estrogen and Tryptophan at Pretreatment Baseline:

At pretreatment baseline, there was a significant positive correlation between levels of estrone and both free ($r(59)=.453$, $p=.001$) and total ($r(61)=.266$, $p=.038$) tryptophan. That is, the higher the level of plasma estrone, the higher were the levels of both free and total plasma tryptophan. However, there was no significant correlation between plasma estradiol levels and either free or total plasma tryptophan.

5) Mood: Effect of Estrogen

a) Relationship between Estrogen and Mood at Pretreatment Baseline:

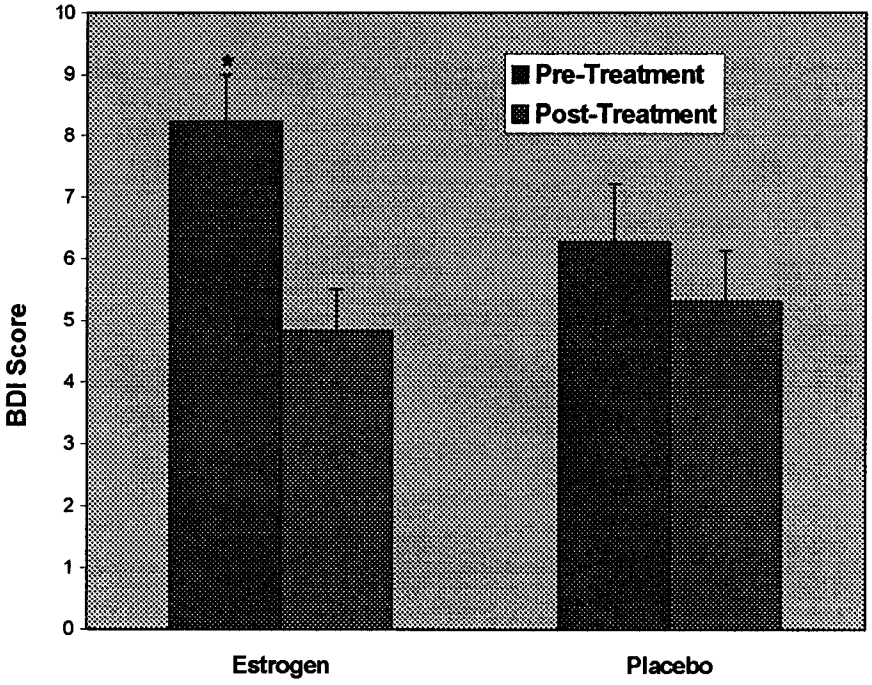
At pretreatment baseline, there was no significant relationship between either plasma estrone ($R(61)=-.079$, $p=.545$) or estradiol levels ($R(61)=-.069$, $p=.596$) and Beck Depression Inventory Scores. However, there was a significant positive relationship between the elation/depression scale of the Profile of Mood States and plasma estrone levels ($R(61)=.300$, $p=.019$), but not plasma estradiol levels ($R(61)=.195$, $p=.132$). Higher levels of estrone were significantly associated with enhanced mood.

b) The Effect of Treatment with Estrogen on Beck Depression Inventory (BDI) Scores:

At pre-treatment baseline, there were no significant differences between the two groups on mean BDI scores (mean = 8.22 (+/- .79) for the Estrogen group and 6.28 (+/- .94) for the Placebo group). Since scores from 0-9 on the BDI are considered to be within the normal range (Beck & Steer, 1987), both groups were euthymic prior to treatment.

In an analysis of the effect of treatment with estrogen or placebo on mood, a two-way mixed ANOVA (with Day as the within group factor and Hormone Status as the between group factor) revealed a significant main effect of Day ($F(1,59=15.17$, $p=.000$) and a significant interaction of Day X Hormone Status ($F(1,59)=4.731$, $p=.034$). Planned comparisons revealed that the mood of estrogen treated women was significantly improved following treatment (pre-treatment mean = 8.22 (+/- .79), post-treatment mean = 4.83 (+/-.68)) ($t(35)=4.571$, $p< .001$) whereas the mood of placebo treated women did not change from pre- to post-treatment (pre-treatment mean = 6.28 (+/- .94), post-treatment mean = 5.32) (figure 8).

Figure 8: Effects of Estrogen or Placebo Treatment on Beck Depression Inventory Scores (BDI)



Treatment with estrogen (Premarin .625 mg/day for 12 weeks) was compared to treatment with placebo for the same time period. Repeated measures ANOVA revealed a significant main effect of Day ($p<001$) and a significant interaction of Day X Treatment ($p=.034$). Estrogen treated women ($n=36$) demonstrated a significant improvement in mood as measured by the BDI ($p<.001$). The mood of placebo treated women ($n=25$) did not change significantly.

c) Effect of Treatment with Estrogen or Placebo on Profile of Mood States (POMS) scores:

At pre-treatment baseline, the mean POMS Scores of 26.28 (+/- .92) for the Estrogen group and 25.96 (+/- 1.951) for the Placebo group were not significantly different (t (1,59)=.190, p=.850).

In an analysis of the effect of treatment on POMS scores, a two-way mixed ANOVA (with Day as the within group factor and Hormone Status as the between group factor) failed to reveal a significant main effect of Day or a significant interaction of Day X Hormone Status.

6) Mood: Effect of Tryptophan Depletion

a) Drink Administration Order Effect:

Order of drink presentation (tryptophan deficient drink (T-) day and balanced drink (B) day) was random, double-blind and counterbalanced between groups. A 2-way mixed ANOVA of BDI change scores using Order as the between subjects factor and Drink as the repeated measure revealed no main effect of Order (F(1,56)=.009, p=.925) or interaction of Drink X Order (F=(1,56)=.078, p=.781). Since no effect of Drink order was found, order was not considered in subsequent analyses.

b) Effect of Drink Status on Overall Mood Change as Measured by the POMS:

Change scores for POMS data were calculated by subtracting Post ATD scores (five hours following ingestion of the amino acid drink) from Pre ATD scores (prior to ingestion of the amino acid drink). This was done in order to control for intra-subject variability. Two-way mixed ANOVAs (with Drink as the within group factor and Hormone Status as the between factor) on POMS data failed to reveal any significant effects of tryptophan depletion on any of the POMS subscales including Elated/ Depressed, Clearheaded/Confused, Energetic/Tired, Confident/Unsure, Calm/Anxious, and Agreeable/Hostile.

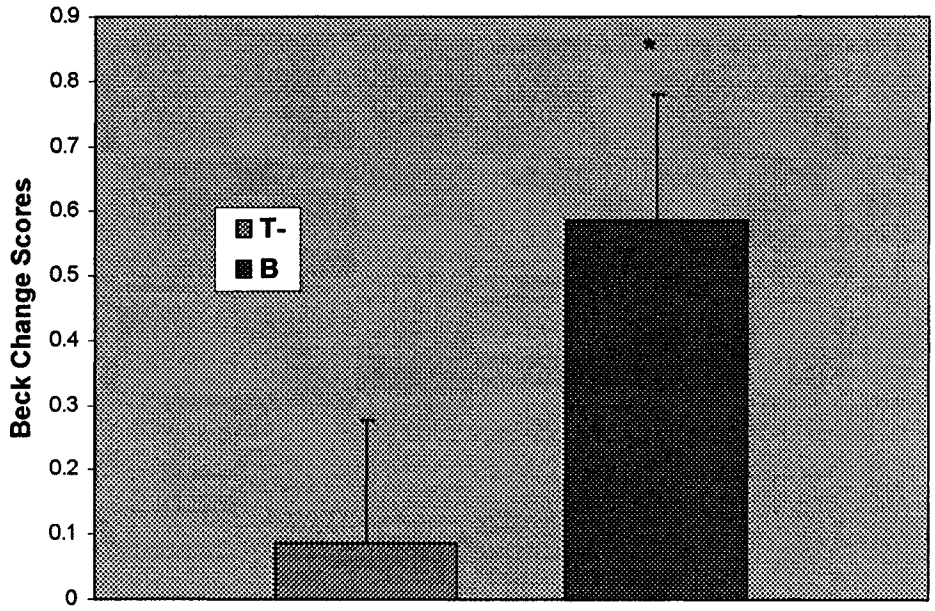
c) Effect of Drink Status on Overall Mood Change as measured by the BDI:

Change scores for BDI data were also calculated by subtracting Post ATD scores (five hours following ingestion of the amino acid drink) from Pre ATD scores (prior to ingestion of the amino acid drink) on both test days, to control for intra-subject variability.

A two-way mixed ANOVA undertaken to assess the effect of ATD on mood revealed a significant main effect of Drink ($F(1,56)=4.763$, $p=.033$), but no main effect of Hormone Status and no interaction effect of Drink X Hormone Status. Thus while tryptophan depletion significantly lowered mood, these results failed to confirm that

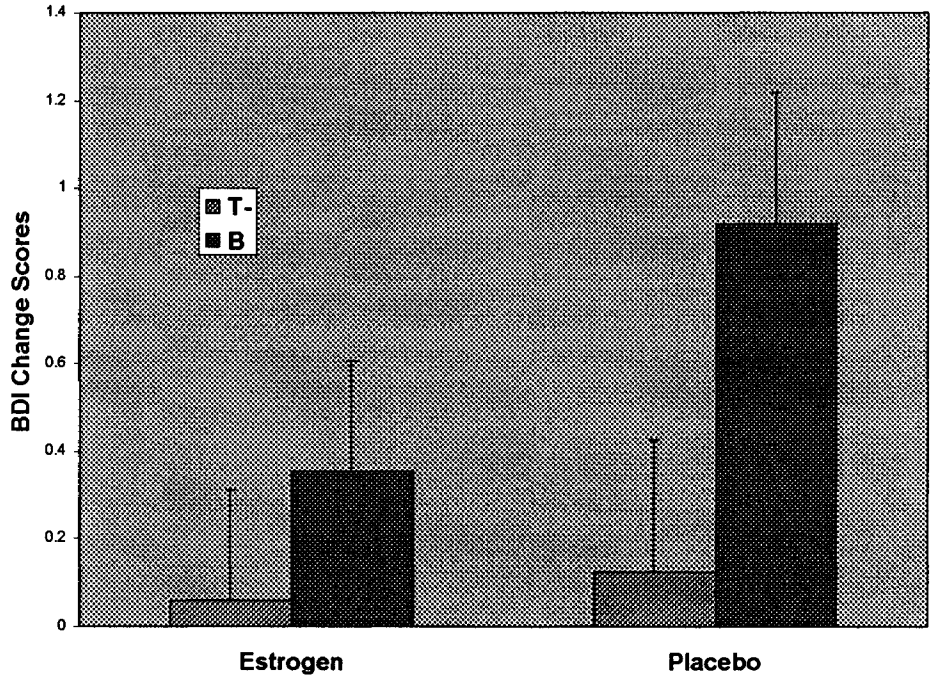
hormone status was influential in the mood-lowering effect of tryptophan depletion (figures 9a and 9b).

Figure 9a: Effect of Acute Tryptophan Depletion on Overall Beck Depression Inventory Score



Bars represent scores (expressed as change from baseline) on the Beck Depression Inventory on 2 test days (T- =Tryptophan deficient, B=Balanced Amino Acid drink). Repeated measures ANOVA (n=58) reveals a significant main effect of Test Day (p=.033). This indicates that menopausal women show a small, but statistically significant mood reaction to ATD such that the T- drink is associated with less positive mood change than the B drink.

Figure 9b: Effect of Hormone Treatment on Mood Response to ATD



Bars represent scores (expressed as change from baseline) on the Beck Depression Inventory on 2 test days (T- =Tryptophan deficient, B=Balanced Amino Acid drink). While repeated measures ANOVA (n=58) reveals a significant main effect of Test Day, there is no main effect of hormone treatment, and no significant interaction effect.

d) Analysis of Individual Items on the BDI:

Since the BDI change score revealed a significant effect of ATD, but the POMS failed to find a change in mood following tryptophan depletion, the question remained as to what aspects of mood changed on the BDI following tryptophan depletion. The BDI taps into several elements of depressive affect that may differ from those measured by the POMS. The following analyses therefore examined which specific items on the BDI were significantly affected by tryptophan depletion.

In the following analyses, the data for one subject were not available, leaving 57 participants in total. Two-way mixed ANOVAs (with Drink as the within group factor and Hormone Status as the between group factor) on BDI individual item change scores revealed that the item which assesses general feelings of sadness (Q1) was significantly affected by tryptophan depletion (significant main effect of Drink, $F(1, 55)=7.545$, $p=.008$). Participants reported feeling more sad following ingestion of the T- drink (mean change score =-.007 (+/-.003)) than following ingestion of the B drink (mean change score = .005 (+/-.002)). The item assessing feelings of failure (Q3) was also significantly affected by tryptophan depletion (significant main effect of Drink, $F(1,55)=4.265$, $p=.044$). Participants reported greater feelings of failure following ingestion of the T- drink (mean change score = -.001 (+/-.001)) than following ingestion of the B drink (mean change score = .005 (+/- .002). There was a trend towards significance on the item assessing general satisfaction (Q4) ($F(1,55)=3.521$, $p=.066$).

Participants reported feeling less satisfied following the T- drink (mean change score =-.005 (+/-.005)) than following the B drink (mean change score =.005 (+/-.003)). None of the other items changed significantly based on drink status.

e) Overall Mood Change as measured by the Visual Analogue Mood Scale (VAMS):

Change scores for VAMS data were calculated by subtracting P.M. post-scores (five hours following ingestion of the amino acid drink) from A.M. pre-scores (prior to ingestion of the amino acid drink). This was done in order to control for intra-subject variability. Two-way mixed ANOVAs (with Drink as the within group factor and Hormone Status as the between factor) on VAMS data failed to reveal any significant effects of tryptophan depletion on any of the following dimensions: Alert/Drowsy, Calm/Excited, Strong/Feeble, Muzzy/Clear-headed, Lethargic/Energetic, Contented/Discontented, Mentally Slow/Quick-witted, Tense/Relaxed, Attentive/Dreamy, Incompetent/Proficient, Happy/Sad, Antagonistic/Amicable, Interested/Bored, Withdrawn/Gregarious, Hungry/Full. On the dimension Coordinated/Clumsy, the T- drink was associated with significantly greater feelings of clumsiness (mean=-1.555 (+/-.46)) than the B drink (mean=-459 (+/-.22)) (main effect of Drink, $F(1,42)=4.783$, $p=.034$). In addition, there was a trend towards a significant main effect of Drink on the Troubled/Tranquil dimension ($F(1,42)=3.289$, $p=.077$). The T-drink was associated with increases in troubled feelings (mean=.415), while the B drink was associated with decreases in troubled feelings (mean = -.326).

f) Effect of Personal History of Affective Disturbance:

Of the 58 women who completed the study, 16 (28%) had a past history of affective disturbance as defined by DSM-IV diagnostic criteria and as measured by the SCID. A two-way mixed ANOVA with Drink as the within subject factor and personal history of affective disturbance as the between subject factor revealed a significant main effect of Drink ($F(1,56)=5.394$, $p=.024$) but no main effect of History ($F(1,56)=1.005$, $p=.320$) and no interaction effect of Drink X History ($F(1,56)=1.219$, $p=.274$). Thus, there is no evidence that past history of affective disturbance exerted an influence on response to ATD in this population. However, it is noteworthy that the two groups tended to respond in a different manner to the two drinks. In response to the T- drink, the mood of women with a past history of affective disturbance worsened (mean change score = -.375 (+/- .360)) while in response to the B drink, it improved (mean change score = .563 (+/-.375). On the other hand, women without a history of affective disturbance responded with improved mood to both the T- drink (mean change score = .262 (+/-.222)) and to the B drink (mean change score = .595 (+/-.232).

g) Effect of Personal History of Reproduction-related Affective Disturbance:

The following analyses examine the role of a personal history of reproduction-related affective disturbance in mood response to tryptophan depletion.

i) Premenstrual dysphoric disorder (PMDD):

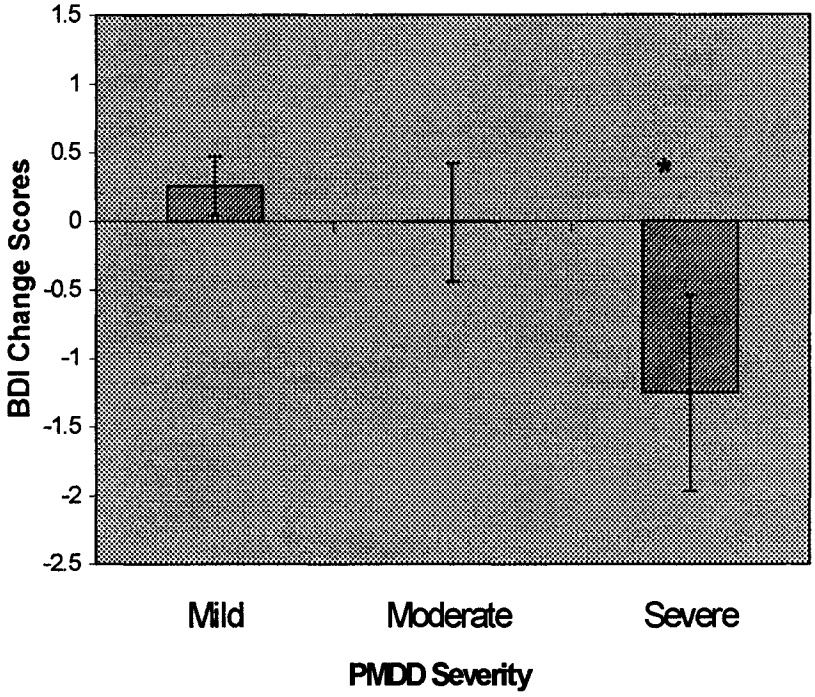
The diagnosis of a past history of severe, moderate or mild Premenstrual Dysphoric Disorder was based on DSM-IV research criteria. Severe PMDD was diagnosed when a participant met all clinical criteria for PMDD, including social and/or occupational repercussions. To be classified as moderate PMDD, participants had to report significant symptoms whose intensity did not meet diagnostic criteria for >5 "severe" or "incapacitating" symptoms, whose symptoms were not frequent enough to meet criteria, or whose symptoms did not interfere with life functioning. Participants classified as having no history of PMDD reported either no symptoms, barely noticeable symptoms or mild symptoms that did not affect life functioning. Using the BDI as the dependent measure, women who reported a history of severe premenstrual symptoms (n=4) responded differently to the mood lowering effects of tryptophan depletion than did those who reported moderate (n=11) or no symptoms (n=43) (figure 10). A two-way mixed ANOVA with History of PMDD as the between subjects factor and Drink as the within subjects factor revealed a significant effect of Drink ($F(1,55)=8.991$, $p=.004$) and a significant interaction of History X Drink ($F(1,55)=3.527$, $p= .036$). Planned comparisons revealed that the mood of women with a history of severe PMDD (as measured by the BDI) was lowered to a significantly greater extent following tryptophan depletion (mean change score = -1.250 (+/-.713) than that of women with mild or no PMDD (mean change score = .256 (+/-.217) ($t(45)=2.122$, $p=.039$). The mood of women with a history of moderate PMDD (mean change score = -.009 (+/-.430) fell between those with severe symptoms and those with no PMDD symptoms following the T-

treatment. However, these differences did not reach conventional levels of statistical significance.

Whereas in absolute terms, women with a history of PMDD had greater mood elevation following the B drink (mean change score = 1.5 (+/-.735) than those with a moderate (mean change score = .000 (+/- .443) or no history (mean change score = .651 (+/-.224), none of these differences attained statistical significance.

Overall, these results suggest that women with a history of PMDD may be more sensitive to the mood lowering effects of tryptophan depletion.

Figure 10: Effect of Premenstrual Dysphoric Disorder Severity on Mood Lowering Following Tryptophan Depletion



Two-way mixed ANOVA with History of PMDD as the between subjects factor and Test Day as the within subjects factor revealed a significant effect of Test Day ($F(1,55)=8.991$, $p=.004$) and a significant interaction of History X Test Day ($F(1,55)=3.527$, $p=.036$). The mood of women who report severe symptoms of PMDD was lowered to a significantly greater extent following tryptophan depletion than that of women who reported mild or no symptoms.

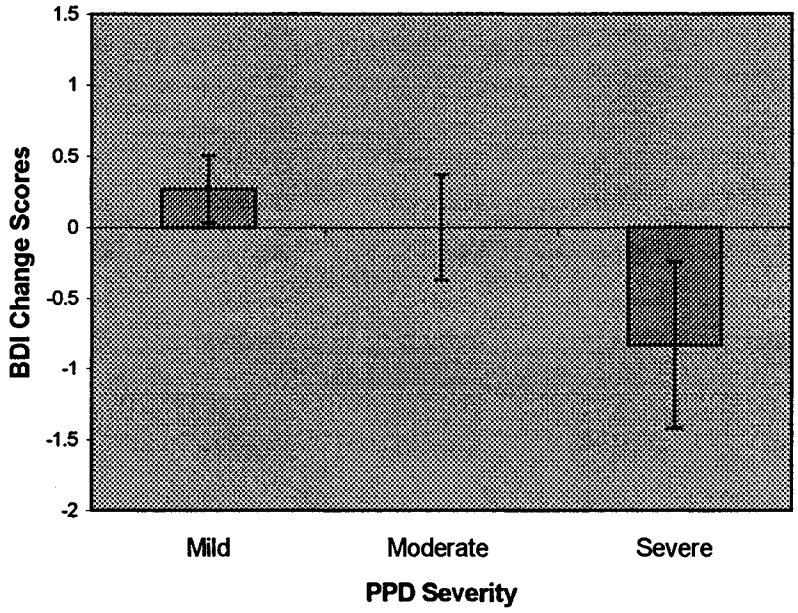
ii) Postpartum Depression (PPD):

Postpartum depression was defined according to DSM-IV diagnostic criteria and measured using the SCID. Participants who met all criteria for PPD were categorized as "severe", those who had significant symptoms but did not meet diagnostic criteria were categorized as "moderate" and those with only mild (postpartum blues) or no postpartum symptoms were categorized as having no history.

A similar pattern to that seen in women with premenstrual dysphoric disorder occurred when those who retrospectively reported a history of severe postpartum depression (n=6) were compared to those who reported moderate symptoms (n=15) or no history (n=37) of PPD. However, these differences failed to reach statistical significance (figure 11). Two way mixed ANOVA with History of PPD as the between group factor and Drink as the within group factor revealed only a significant main effect of Drink ($F(1,55)=8.453$, $p=.005$). However, there was a trend for greater mood lowering in response to the T- drink in women who reported a history of severe PPD (mean change score = -.833 (+/-.588) compared to women who reported a moderate history (mean change score = .00 (+/-.372) or no history (mean change score = .270 (+/- .237) as indicated by the interaction effect of PPD History X Drink ($F(1,55)=2.959$) $p=.061$).

In response to the B drink, women with a history of severe PPD reported greater mood elevation (1.33 (+/-.602)) than women with a moderate history (.133 (+/-.381)) or no history (.649 (+/-.242), but once again, these differences failed to reach statistical significance.

Figure 11: Effect of Postpartum Depression Severity and on Mood Lowering Following Tryptophan Depletion



Two way mixed ANOVA with PPD History as the between group factor and Test Day as the within group factor revealed only a significant main effect of day ($F(1,55)=8.453$, $p=.005$). However, there was a trend for greater mood lowering in response to the T- drink in women with a history of severe PPD as compared to women with a moderate history or no history as indicated by the interaction effect ($F(1,55)=2.959$) $p=.061$).

iii) Overall History of Reproduction-related Affective Disturbance:

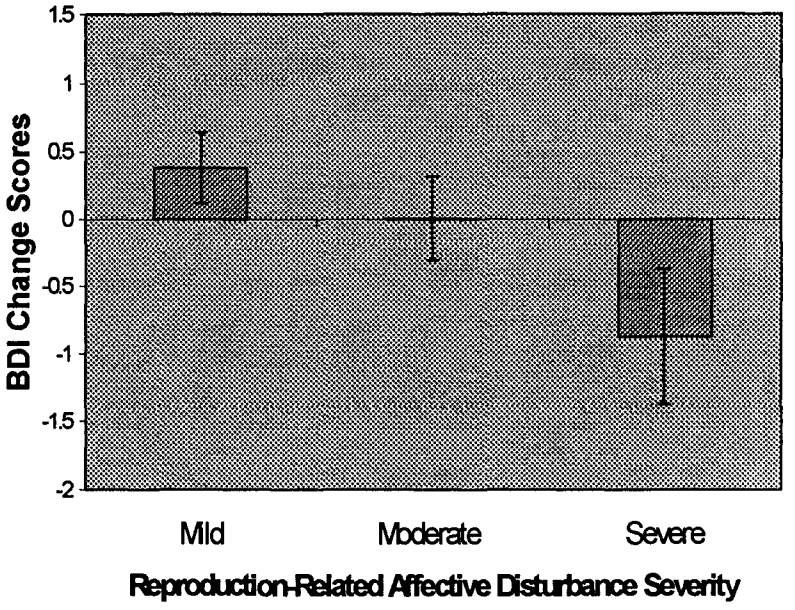
In order to examine the effect of *any* type of reproduction-related affective disturbance on the mood lowering effect of tryptophan depletion, three groups where created: 1) no history of PMDD or PPD (n=29), 2) moderate history of PMDD and/or PPD, (n=21), 3) severe history of PMDD and/or PPD (n=8). Using BDI scores as the dependent variable, again, a similar pattern occurred (figure 12). A two-way mixed ANOVA with History of reproduction-related affective disturbance as the between groups factor and Drink as the within groups factor revealed a main effect of Drink ($F(1,55)=9.907$, $p=.003$) and a significant interaction of History X Drink ($F(1,55)=4.423$, $p=.017$). Planned comparisons revealed that women with a history of severe reproduction-related affective disturbance reported significantly greater mood lowering in response to the T- drink (mean change score = -.875 (+/-.501)) than did women with no history of reproduction-related affective disturbance (mean change score = .379 (+/-.263)) ($t(35)=2.108$, $p=.042$). The mood scores of women with a moderate history of reproduction-related affective disturbance (mean change score = .004 (+/- .310) fell between those of women with a severe history and no history, but these differences were not statistically significant.

In addition, women who reported severe reproduction-related affective disturbance symptoms demonstrated significantly greater positive mood change on the B day (mean change score = 1.25 (+/- .508) compared to women with a moderate history of reproduction-related affective disturbance (mean change score = .000 (+/- .314) ($t(27)=-$

2.318, p=.028). However, their mood did not differ from women with no history of reproduction-related affective disturbance (mean change score = .828 (+/- .267)).

Overall, these data suggest that women with a history of severe reproduction-related affective disturbance are more vulnerable to tryptophan depletion induced mood lowering then women with a mild or with no history of such disorders.

Figure 12: Effect of Reproduction-Related Affective Disturbance Severity on Mood Lowering Following Tryptophan Depletion



Two way mixed ANOVA with History of Reproduction Related Affective Disturbance as the between groups factor and Test Day as the within groups factor revealed a main effect of Test Day ($F(1,55)=9.907$, $p=.003$) and a significant interaction of History X Day ($F(1,55)=4.423$, $p=.017$). Planned comparisons revealed that women with a severe history of severe reported significantly greater mood lowering in response to the T- drink (mean change score = -.875 (+/-.501)) than did women who had no history (mean change score = .379 (+/-.263)) ($t(35)=2.108$, $p=.042$).

h) Effect of Family History of Affective Disturbance

A participant was deemed to have had a family history of affective disturbance if at least one first degree relative had a clear history of Major Depressive Disorder based on retrospective reports of the participants that were gathered by means of the Family Instrument for Genetic Studies (FIGS) (Nurnberger et al, 1994). Using these criteria, 22 of 56 participants (data missing for two participants) were classified as having a family history of affective disturbance. A two-way mixed ANOVA with Drink as the within group factor and Family History of Affective Disturbance as the between group factor revealed no main effect of Family History of Affective Disturbance and no significant interaction of Family History of Affective Disturbance X Drink. There is therefore no evidence that a family history of affective disturbance was associated with a greater response to tryptophan depletion in this population.

I) Effect of Family History of Alcoholism:

A participant was deemed to have had a family history of alcoholism if at least one first degree relative had a clear history of alcoholism based on retrospective reports of the participants gathered using the FIGS. Using these criteria, 17 of 56 participants (data missing for two participants) were classified as having a family history of alcoholism. Two way mixed ANOVA with Drink as within subject factor and Family History of Alcoholism as the between subject factor failed to find evidence that a family

history of alcoholism was associated with greater response to tryptophan depletion in this population. There was no main effect of Family History of Alcoholism and no significant interaction of Family History of Alcoholism X Drink.

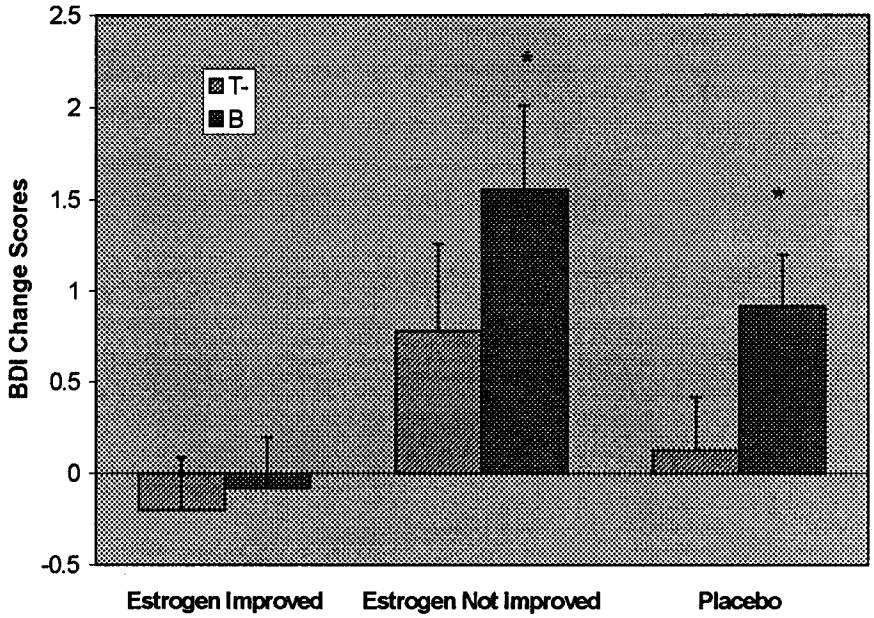
j) Estrogen-Improved vs. Estrogen Not-Improved:

Because not all estrogen treated participants reported an improvement in mood following twelve weeks of treatment with exogenous estrogen, it was hypothesized that there might exist a subset of women who were more (or less) sensitive to the mood altering effects of estrogen. These differences in sensitivity might translate into differing responses to ATD in women treated with estrogen. Therefore, while we did not see evidence of an overall influence of hormonal status on acute tryptophan depletion, it still remained a possibility that the subset of women who were sensitive to the mood enhancing properties of estrogen might respond differently to ATD than women who had no such sensitivity and than women treated with placebo.

In order to elucidate further the possible role of estrogen in the mood lowering effect of ATD, the Estrogen group was divided into two subgroups. These groups were created based on the presence (Estrogen Improved [E-I], n=25) or absence (Estrogen Not Improved [E-N], n=9) of a treatment response to exogenous estrogen as measured by BDI change scores. These two groups were both compared to the placebo-treated group (n=24). A two-way mixed ANOVA revealed a significant effect of Drink ($F(1, 55)=4.238$, $p=.044$) and Group ($F(2, 55)=5.723$, $p=.006$). Tukey's post hoc tests revealed

that the Estrogen Improved group had higher BDI scores on the Balanced drink day than both the Estrogen Not Improved group ($p=.009$) and the Placebo group ($p=.037$). Therefore, women whose mood had improved as a result of estrogen treatment (E-I) showed less variability in mood than the other two groups (figure 13).

Figure 13: Effect of Acute Tryptophan Depletion on BDI Change Scores: Estrogen-Improved (E-I) Participants vs. Estrogen-Not improved (E-N) or Placebo (P) Participants



Bars represent scores (expressed as change from baseline) on the BDI. The two Estrogen groups were created based on the presence (E-I, n=25) or absence (E-N, n=9) of a treatment response to exogenous estrogen over a 3 month period. They were both compared to a placebo-treated group (n=24). Repeated Measures ANOVA revealed a significant effect of Day (p=.044) andTreatment (p=.006). Tukey's post hoc tests revealed significant differences between E-I and both E-N (p=.009) and P (p=.037) on the Balanced drink day.

DISCUSSION

The first hypothesis of this study, which predicted that postmenopausal women would be vulnerable to the mood lowering effects of ATD, was supported. In this sample, ATD caused a significant lowering of mood (as measured by the BDI) compared to the administration of a balanced amino acid drink. This is consistent with the past research which suggested that female gender itself is a vulnerability factor to the mood lowering effects of ATD (Ellenbogen et al., 1996). Conversely, several studies found that males do not respond to ATD unless they have some other vulnerability factor. For example, euthymic males were unresponsive to ATD (Abbott et al., 1992) unless they had a family history of major depressive disorder (Benkelfat et al., 1994) or high-normal baseline depression scores (Young et al., 1985). However, in our sample of women, mood lowering occurred in response to ATD in those without any of these additional vulnerability factors. The relationship between family history of depression and greater response to ATD was not confirmed in this sample of postmenopausal women. These findings are inconsistent with those of Benkelfat (1994) who reported that healthy male participants with a family history of MDD experienced a significantly greater mood lowering following ATD than healthy males with a negative family history of MDD. This suggests that there is a sex difference in responsiveness to a genetic predisposition to depression. That is, a family history of depression does not increase vulnerability to mood lowering in response to ATD in women, but it appears to in men. Future studies

should examine this issue in greater detail in order to gain further understanding of potential sex differences in genetic contributions to vulnerability to depression.

That women are more responsive to a physiological mood lowering manipulation than males is not surprising given the consistent sex difference in the incidence of MDD (Gater et al., 1998; Kessler et al., 1993; Stephens et al., 1999; Weissman et al., 1984) which, in turn, may be related to underlying sex differences in the neuroendocrine system.

The second major hypothesis of this study was that treatment with exogenous estrogen would contribute to maintaining a euthymic mood state in postmenopausal women (Ditkoff, Crary, Cristo, & Lobo, 1991; Sherwin, 1988; Sherwin & Gelfand, 1985), thereby providing some protection against the mood-lowering effects of the ATD procedure. While the postmenopausal women in this study responded to ATD with lowered mood, and while treatment with exogenous estrogen enhanced participants' mood, treatment with exogenous estrogen did not significantly protect women from the mood lowering associated with ATD. Thus our initial hypothesis that estrogen would exert a protective effect with respect to the mood lowering of ATD was not confirmed. Despite the fact that treatment with exogenous estrogen for eleven weeks improved mood in this sample of postmenopausal women, estrogen treatment did not prevent or attenuate the challenge presented by the acute serotonergic manipulation employed in this study. This suggests that a low estrogen state, in itself, is not a vulnerability factor for the dysphoric mood changes caused by ATD and does not provide further evidence for the influence of estrogen on the serotonergic system (Fink et al., 1996; McEwen, 1994).

Moreover, these data suggest that it is not absolute levels of hormones that constitute a vulnerability to mood-lowering in response to ATD in women, but rather that the fluctuations in reproductive hormones that occur during reproductive events across the life cycle may be responsible for creating a greater vulnerability to ATD mood effects in women.

Like all psychopharmacologic agents, there is considerable variability in the mood response to hormones, including to exogenous estrogen. That is, some women respond to treatment with exogenous estrogen with mood enhancement while others do not (Ditkoff et al., 1991; Schmidt et al., 2000; Sherwin, 1988; Sherwin & Gelfand, 1985; Soares et al., 2001). If women have particular sensitivities to estrogen and its effects, it is reasonable to speculate that they may respond differently to ATD based on their response to treatment with exogenous estrogen. Therefore, another major hypothesis of this study was that regardless of the overall effect of estrogen status on the mood-lowering effect of ATD, there would exist a subsample of women who were more susceptible, or sensitive, to the effects of estrogen on mood who would also respond differentially to ATD compared to women without such a sensitivity. Sensitivity to estrogen was operationally defined as enhanced mood in response to 11 weeks of treatment with estrogen. There is currently no chemical index that would substitute for clinical response. We postulated that women who were sensitive to the mood enhancing effects of exogenous estrogen would benefit to a greater degree from estrogen's postulated protective effects following ATD than women who were less sensitive to estrogen's mood effects. This hypothesis was confirmed. When participants were grouped according to the presence or absence of

estrogen-induced changes in mood during the eleven-week treatment phase of the study, those whose mood had improved with estrogen treatment demonstrated no ATD mood effect, whereas those who did not respond to estrogen with improved mood experienced an ATD mood effect. In other words, women who responded to estrogen with enhanced mood showed less variability in their mood in response to ATD than those who did not respond to estrogen with enhanced mood or those treated with placebo. Thus, in women who were sensitive to the mood enhancing properties of estrogen, estrogen treatment may have been protective with regard to the mood-lowering ATD effect. While these data do not provide evidence of estrogen's effects on the serotonergic system overall, they do suggest an interaction between estrogen and serotonin in a subpopulation of women who are sensitive to the mood-enhancing properties of estrogen.

The kindling model of depression suggests that the experience of a given depressive episode reduces the threshold for an individual's neural circuitry to enter into a future depressive state. Eventually, the threshold is lowered to such an extent that episodes occur spontaneously, without an obvious environmental stressor (Kendler et al., 2001; Post, 1992). The kindling model of depression also holds that prior experience with mood changes associated with naturally occurring fluctuations in reproductive hormones (such as premenstrual or postpartum mood changes) might sensitize women to future depressive episodes in response to environmental or physiological stressors (Halbreich, 2000; Parry, 1992, 1995). Therefore, the final major hypothesis of this study was that a past history of affective disturbance, particularly reproduction-related affective disturbance, would predict a more profound mood response to ATD. More than a quarter

(28%) of the women who participated in the study had a past history of depression as defined by DSM-IV diagnostic criteria. No significant relationships between response to ATD and history of MDD were noted regardless of hormone status. While these data do not support the prediction that past history of major affective disturbance influences the mood lowering effect of ATD in this population, there is evidence that reproduction-related affective disturbance modifies response to ATD. Participants who reported a past history of severe premenstrual symptoms (severe PMDD) responded differently to the mood lowering effects of ATD than those who reported moderate or no symptoms ($p<.036$). That is, those with a history of severe PMDD had significantly lower mood following ingestion of the T-drink than those with mild or no symptoms of PMDD. The mood scores of those who reported moderate symptoms fell in between the other two groups with respect to response to tryptophan depletion, although this difference failed to reach conventional levels of statistical significance. This suggests that women with a history of PMDD may be more sensitive to the mood lowering effects of tryptophan depletion than those without such a history. A similar pattern was noted in women with a history of PPD but the relationship failed to reach conventional standards of statistical significance ($p<.06$). However, when all women with a history of reproduction-related mood disorders were examined together, the effect was statistically significant ($p<.017$) implying that women with a history of severe reproduction-related affective disturbance are more vulnerable to tryptophan depletion induced mood change than those with a mild or with no history of prior reproduction-related affective disturbance. It is possible that these women are more sensitive to fluctuations in tryptophan and/or serotonin levels in general, which might be a potential mechanism for their vulnerability to depression.

While the exact mechanism of this greater vulnerability has not been determined there is considerable evidence that PMDD and PPD are both associated with differences in the serotonergic system. For example, free and total plasma tryptophan levels have been shown to be negatively correlated with postpartum mood (Gard, Handley, Parsons, & Waldron, 1986; Stein, Milton, Bebbington, Wood, & Coppen, 1976; Handley, Dunn, Baker, Cockshott, & Gould, 1977) and decreased uptake and lower levels of serotonin in platelets have been reported in PMDD participants as compared to controls (Ashby et al., 1988) (Ashby et al., 1988; Gard et al., 1986; Handley et al., 1977; Hannah et al., 1992; Maes et al., 1992; Stein et al., 1976; Steiner, 1997; Taylor et al., 1984). The results of this study are consistent with the kindling model of depression which postulates that a past history of mood disturbance makes one more likely to experience future episodes of mood change in response to biological or environmental stressors or challenges (Halbreich, 2000; Parry, 1992, 1995).

Other results of this study either confirm those of other researchers or provide novel findings with regard to estrogen and mood in women. At pretreatment (prior to administration of estrogen or placebo), no significant relationship between either plasma estrone or estradiol levels and mood (as measured by the BDI) was seen in this group of postmenopausal women. However, a significant positive relationship between the elation/depression scale of the Profile of Mood States and plasma estrone levels occurred such that higher levels of estrone were associated with more positive mood. This was consistent with the evidence that higher estrogen levels are associated with enhanced mood in postmenopausal women (Ditkoff et al., 1991; Sherwin, 1988; Sherwin &

Gelfand, 1985). This may have occurred because estrone is the dominant estrogen in postmenopausal women. It is produced in fat cells via conversion from other steroid hormone precursors so that its production endures after the ovaries become atrophic.

It was also the case that a significant positive relationship between baseline levels of estrone and both free and total plasma tryptophan levels occurred (prior to treatment with exogenous estrogen or placebo). A similar relationship between free plasma tryptophan and plasma estrone, but not between total plasma tryptophan and plasma estrone, has been reported previously (Aylward, 1976; Aylward & Maddock, 1973; Thomson et al., 1977). Unlike other investigators, we found no relationship between estradiol and tryptophan levels and no effect of exogenous estrogen on tryptophan levels. The lack of a relationship between estradiol and tryptophan levels may be related to the fact that estradiol levels are extremely low in postmenopausal women and, as mentioned earlier, estrone is the dominant estrogen in menopause. That the relationship between estrone levels and free plasma tryptophan was stronger than that between estrone and total plasma tryptophan is understandable given that estrogen competes with plasma tryptophan for binding to plasma albumin (Aylward, 1973). Thus, the higher the level of estrogen, the greater the expected amount of free tryptophan. This finding confirms that estrogen could potentially exert its effects on serotonin and mood via tryptophan availability.

Compliance and efficacy of treatments:

Hormone Treatment:

At pretreatment baseline, all participants had levels of follicle stimulating hormone, luteneizing hormone, estradiol and estrone within the postmenopausal range. The two groups did not differ significantly on any of the hormone measures, confirming that they had a similar hormonal milieu prior to administration of exogenous estrogen or placebo. Following eleven weeks of treatment with estrogen, plasma estradiol and estrone values increased significantly compared to both pretreatment baseline levels and to placebo control levels, confirming participants' compliance with the medication regimen and the effectiveness of the hormonal manipulation.

Treatment with exogenous estrogen significantly reduced hot flush frequency compared with placebo treatment. A similar pattern was seen with respect to cold sweats, although this difference failed to reach statistical significance. Both groups reported significantly decreased frequency of symptoms from pre- to post-treatment as measured by the total score on the Menopausal Index, suggesting a relatively powerful placebo effect. That no overall effect of estrogen treatment was found on the Menopausal Index is not surprising given the range of symptoms evaluated with this measure (e.g. "feelings of suffocation", "rheumatic pains", "weight gain"). Given the significant difference in the

most prevalent symptom of the menopause, namely hot flushes, it can be concluded that treatment with exogenous estrogen exerted the expected physiological effects.

As expected, treatment with exogenous estrogen for eleven weeks resulted in a significant improvement in mood as measured by the BDI. This is consistent with the literature on estrogen and mood in healthy women without current diagnoses of MDD (Campbell, 1976; Carranza-Lira & Valentino-Figueroa, 1999; Dennerstein, Burrows, Hyman, & Sharpe, 1979; Ditkoff et al., 1991; Sherwin, 1988; Sherwin & Gelfand, 1985; Sherwin & Suranyi-Cadotte, 1990).

Acute Tryptophan Depletion:

At pretreatment baseline (prior to any experimental intervention), there were no group differences in levels of free or total plasma tryptophan. The same was true on ATD test days prior to participants' ingestion of the amino acid drinks (e.g. there were no differences in A.M. baseline levels of free and total plasma tryptophan between the estrogen and placebo groups). That there were no differences in plasma tryptophan levels following treatment with estrogen is not consistent with previous findings (Aylward, 1973; Bender et al., 1983), but may reflect the rigorous dietary requirements of the present study prior to and on tryptophan depletion test days. The low protein diet and overnight fast that all participants followed in the 24 hours prior to test days alter plasma tryptophan levels such that they would not reflect the levels that would be seen in individuals with unrestricted dietary intake.

ATD resulted in an 86% reduction in total plasma tryptophan levels, while administration of the balanced drink increased levels by 187%. A similar pattern was noted with respect to free plasma tryptophan (an 82% decrease following ATD and a 209% increase following administration of the balanced amino acid drink). These differences were statistically significant and consistent with the effects reported by others (Young et al., 1989). Moreover, it is likely that these changes in total and free plasma tryptophan reflect decreases in brain tryptophan and serotonin levels. This is due to the fact that brain tryptophan hydroxylase is only approximately 50% saturated with tryptophan in humans, suggesting that the decline in the availability of tryptophan produced by tryptophan depletion caused a lowering of brain tryptophan (Young & Gauthier, 1981). That this decrease in brain serotonin occurs following ATD has been confirmed by evidence of declines in both serotonin synthesis and CSF 5-HIAA following ATD in humans (Carpenter et al., 1998; Nishizawa et al., 1997; Williams et al., 1999).

As already discussed, ATD caused a significant lowering of mood in postmenopausal women in this study. Although the specific pattern of results found with regard to the mood lowering effects of ATD is not unusual (Young, 2001 – personal communication), it is worthy of comment. It appears that administration of the balanced drink caused mood to be enhanced rather than maintained whereas the T- drink prevented that mood enhancement. One possible explanation of this finding is that when participants arrive for a test session, they may be somewhat apprehensive. However, the

test day is a for the most part a pleasant and non-demanding experience, thus naturally enhancing mood. Tryptophan depletion, then, simply prevented the mood enhancement that would have been expected. It is highly unlikely that ingestion of the exogenous tryptophan contained in the balanced amino acid drink resulted in enhanced mood. While plasma levels of tryptophan were increased following the B drink, brain levels of tryptophan were likely unaffected. This is due to the fact that the presence of other large neutral amino acids in the B drink establishes competition for transport into the brain resulting in no net increase in brain tryptophan (Fernstrom & Wurtman, 1971). Therefore, the most likely explanation for the enhanced mood is, as described, the generally pleasant nature of the test day.

Validity and reliability of measures:

While scores on the BDI increased significantly following ATD, scores on the POMS did not change significantly following administration of the amino acid drinks. This is somewhat surprising given that the POMS is thought to be a measure that is sensitive to acute changes in mood, particularly drug-induced mood changes. The BDI, on the other hand, is a clinical measure with several items assessing vegetative symptoms that could not easily change over the course of a day (thus decreasing the chances of a significant effect and providing a more conservative measure of mood change). The results of the VAMS were not entirely consistent with those of the BDI either in that some items assessing mood did not change significantly following ATD. However, they did show a significant effect of ATD on feelings of clumsiness. This is not something which has been noted in the past and might be worthy of further investigation,

particularly as serotonergic activity has been positively associated with movement and general behavioral arousal (Jacobs, 1991; Jacobs & Fornal, 1997). There was also a trend towards a significant effect of ATD on the troubled/tranquil dimension of the VAMS ($p=.077$) (with the T- drink associated with greater "troubled" feelings, consistent with the notion of lowered mood).

Given the discrepancies in the findings between the BDI, the POMS and the VAMS, the question remained as to what aspects of mood, specifically, were changing as a result of administration of the tryptophan deficient and the balanced amino acid drink. In order to examine this question more thoroughly, the effects of the two drinks on the individual items of the BDI were analyzed to determine which were most influenced and thus contributed most to the significant effect. The item assessing general feelings of sadness was significantly affected by ATD ($p<.008$), as was the item assessing feelings of failure ($p<.044$). There was also a trend toward significance with the item assessing general satisfaction ($p<.066$). None of the other items changed significantly. This suggests that ATD did, in fact, influence the affective elements of mood, but not the associated somatic elements such as fatigue or appetite. That feelings of failure were influenced by ATD may be related to the phenomenon of state-dependent recall (that is, the triggering of memories of failure when mood is lowered) (Eich, Macaulay, & Ryan, 1994; Kenealy, 1997).

In summary, the major findings of this study were that, 1) postmenopausal women were vulnerable to the mood lowering effects of ATD, 2) estrogen treatment did not

protect postmenopausal women from this effect overall, 3) a subsample of women who responded to treatment with estrogen with enhanced mood were protected by estrogen from the mood-lowering associated with ATD, and 4) a past history of reproduction-related affective disorders was associated with greater vulnerability to the mood lowering that occurs as a result of ATD.

Study Limitations:

This study was a prospective, double-blind, crossover, placebo-controlled investigation. The rigorous nature of the design of this study contributes significantly to the confidence with which one can regard the findings. Nonetheless, several limitations of this study must be acknowledged.

One of the primary limitations of this study are the small sample sizes used for the secondary analyses. In particular, conclusions drawn from the analysis of the possible role of history of reproduction-related affected disturbance and tryptophan depletion must be interpreted with caution in light of the small and unequal sample sizes. Moreover, these analyses and all those involving personal history of psychopathology are also limited by the retrospective and self-report nature of the diagnoses. Although our diagnostic criteria were extremely conservative and although use of the SCID for retrospective diagnosis is well-accepted by the research community, future studies should try to circumvent some of the issues inherent in retrospective diagnosis. This could be accomplished by obtaining corroboration from other informants or medical charts. It

could also be accomplished by using a prospective design in which women with active symptoms of PMDD or PDD are diagnosed and followed longitudinally until they enter the menopausal transition.

Another important limitation of the study was the use of a single dose of exogenous estrogen (conjugated equine estrogen, 0.625 mg daily) rather than a broader range of doses to determine possible dose-response curves. While the one study that examined the effect of two doses of estrogen on mood in postmenopausal women found no dose-response effect (Ditkoff et al., 1991), the role of different doses of estrogen in the mood-lowering effect of ATD has never been examined. It is possible that higher doses of estrogen may have resulted in a more generalized protective effect of estrogen on mood rather than protecting only the subset of women who responded to estrogen treatment with mood enhancement. Conversely, Parry (1992) has suggested that estrogen may have a limited range (or window) of efficacy that varies as a function of phase of the life cycle. Thus, very high, supraphysiological doses of estrogen may exceed this window of efficacy resulting in no protective effect from ATD for any women. This too may be an important consideration for future research.

Future Research:

The primary focus for future research in this area should include further examinations of the relationship between a past history of reproduction-related affective disturbance and response to ATD in postmenopausal women using larger sample sizes.

This is particularly true given that we were able to observe significant effects of a history of reproduction-related affective disturbance on response to ATD despite the limitation of small sample sizes. Moreover, a larger sample size would allow for the examination of the role of estrogen in response to ATD in women with a history of reproduction-related affective disturbance, which was precluded by the small sample size in the present study. It has been suggested that the recurrent experience of affective disturbance over time may result in compensatory adaptive changes in the brain (Post & Weiss, 1992). The examination of the role of estrogen in response to ATD in women with a history of reproduction-related affective disturbance could assess the extent to which such a history is associated with differential sensitivity to estrogen's protective effects. In addition, the investigation of the extent to which frequency of reproduction-related depressive episodes is related to the degree of mood lowering in women with a history of reproduction-related affective disturbance following ATD could yield important information regarding the development of the kindling effect. That is, such a study could evaluate the manner in which repeated fluctuations in hormones, when associated with depressive episodes, can influence neural systems such that response to mood lowering challenges is exacerbated.

Examination of the effect of ATD in other clinical populations, such as those with current diagnoses of PMDD and/or PPD, could also yield important information regarding the development of kindling related changes in response to mood lowering stressors or challenges. It would also allow for the examination of natural fluctuations in estrogen in these populations and its influence on mood response to ATD. Such a

protocol might involve a longitudinal examination of women who currently meet diagnostic criteria for PMDD or PPD as they enter into the menopausal transition. This would allow for prospective diagnosis (rather than retrospective as was the case in this study) and would also allow for a powerful within-subjects comparison.

Another line of inquiry involves the examination of the effects of ATD in depressed postmenopausal women prior to and subsequent to treatment with estrogen and/or antidepressants. These results could then be compared to past research of ATD effects in remitted, clinically depressed individuals treated with antidepressant medication (Delgado et al., 1999; Delgado et al., 1994) which demonstrated that treated depressed individuals respond to ATD with a clinical relapse. This could provide important information regarding estrogen's antidepressant effects in postmenopausal women (Schmidt et al., 2000; Soares et al., 2001).

While there is considerable need for research examining the etiology and treatment of affective disorders in women (Stahl, 1998), examinations of healthy women's response to natural fluctuations in hormones may also yield important information regarding the role of sex steroids in mood vulnerability. For example, an examination of the effect of ATD in women in the menstrual vs. the follicular phases of the menstrual cycle, in the immediate postpartum period and during treatment with LHRH agonists could increase our understanding of the impact of naturally occurring fluctuations in estrogen on vulnerability to lowered mood. Issues that could be addressed with such an inquiry include the role of estrogen in mood changes in healthy women, the

potential interaction of progesterone and estrogen as they affect mood vulnerability, and the role of cyclicity in the kindling effect prior to the development of severe mood symptoms.

While all women experience fluctuations in estrogen levels, only a proportion of women experience mood changes associated with these fluctuations (Halbreich, 2000). A smaller proportion develop reproduction-related affective disturbance (Georgiopoulos et al., 1999; Hearn et al., 1998; Rivera-Tovar & Frank, 1990). It seems reasonable to speculate that women who develop mood changes concurrent with natural fluctuations of the reproductive hormones have differential neural sensitivity to these hormones than women who do not develop mood changes when their sex hormone levels fluctuate. Therefore, another avenue to pursue in future research involves examination of the subgroup of women who are selectively sensitive to estrogen's effects. In particular, a study with a crossover design, where all participants receive sequential treatment with both placebo and exogenous estrogen, could lend further support to the idea that women sensitive to estrogen's mood enhancing effects are protected from the mood lowering associated with ATD and could further clarify the nature of that response. That is, this alternate design could demonstrate that a subgroup of postmenopausal women (those sensitive to the mood enhancing effects of estrogen) exhibit a differential response to ATD as a function of the presence or absence of exogenous estrogen while another group (those who are not sensitive to the mood enhancing effects of estrogen) respond consistently to ATD regardless of estrogen status. This would rule out the possibility that

women who respond to estrogen are less vulnerable to the effects of ATD regardless of their estrogen status.

In addition, further exploration of the more general characteristics of these two groups of women (estrogen sensitive and non-sensitive) may yield important clinical information. This information could facilitate predictions regarding which patients are most likely to experience mood changes in response to changes in the hormonal milieu. In addition, this type of investigation could provide clues as to which women might be most responsive to the use of estrogen as a means of treating mood symptoms, either alone (Schmidt et al., 2000; Soares et al., 2001) or as an adjunct to traditional antidepressants (Klaiber et al., 1979; Schneider et al., 1997).

Summary and Conclusions:

The findings of this study demonstrate that healthy postmenopausal women are vulnerable to the mood lowering effects of ATD even in the absence of typical vulnerability markers (such as past history of affective disturbance or past family history of affective disturbance). These data confirm the existence of a sex difference in mood vulnerability.

While treatment with exogenous estrogen did not alter the response of postmenopausal women to ATD overall, a subgroup of women being treated with exogenous estrogen were protected from the mood lowering effects of ATD. Women

who responded to treatment with exogenous estrogen with improved mood were protected from the mood changes associated with ATD compared to women who were relatively insensitive to the mood enhancing properties of estrogen. The suggestion that there exists a subgroup of women who are estrogen-sensitive (that is, who respond to estrogen's mood enhancing properties) has several clinical implications. For example, sensitivity to estrogen may prove to be a marker for increased risk of reproduction-related affective disturbance. Moreover, since not all postmenopausal women respond to estrogen augmentation therapy for treatment resistant depression (Klaiber et al., 1979; Schneider et al., 1997), women who are sensitive to estrogen may respond more positively to estrogen augmentation of antidepressant treatment.

In this study, a past history of severe symptoms of a reproduction-related affective disturbance was shown to be associated with greater vulnerability to the mood lowering effects of tryptophan, suggesting a greater vulnerability to biological challenges in these women. This is consistent with the kindling theory of depression, which predicts that past exposure to depressive episodes increases neural sensitivity to future physiological or environmental stressors (Kendler et al., 2001; Post, 1992). This may suggest an important contribution of reproductive hormones in the kindling process (Halbreich, 2000; Parry, 1992, 1995). The investigation of the role of hormones in affective disorders in women and the examination of the impact of reproductive events on this process could have profound implications for our understanding of affective illness and for the development of future prevention and treatment strategies.

There are two major clinical implications of this study. First, if kindling is important in the reproduction-related affective disorders, this implies that early intervention and prevention of symptoms in pre-menopausal women showing reproduction-related mood symptoms is crucial. The second implication of these data is that women with a history of reproduction-related mood symptoms should be monitored closely for signs of depression as they enter the perimenopause. These women may be more vulnerable to the decline in estrogen that occurs at this time and may benefit from treatment with exogenous estrogen.

In conclusion, these data provide further evidence for the role of estrogen in mood regulation and for the role of the reproductive hormones in the kindling process of affective disturbance. Although they do not provide direct evidence for the influence of estrogen on the serotonergic system, they do so indirectly by suggesting that in some women, estrogen treatment has an effect on mood response to ATD, as does a past history of reproduction-related affective disturbance.

REFERENCES

Abbott, F. V., Etienne, P., Franklin, K. B., Morgan, M. J., Sewitch, M. J., & Young, S. N. (1992). Acute tryptophan depletion blocks morphine analgesia in the cold-pressor test in humans. *Psychopharmacology, 108*(1-2), 60-66.

Abou-Saleh, M. T., Ghubash, R., Karim, L., Krymski, M., & Bhai, I. (1998). Hormonal aspects of postpartum depression. *Psychoneuroendocrinology, 23*(5), 465-475.

Ahokas, A., Kaukoranta, J., & Aito, M. (1999). Effect of oestradiol on postpartum depression. *Psychopharmacology, 146*(1), 108-110.

Altshuler, L. L., Hendrick, V., & Parry, B. (1995). Pharmacological management of premenstrual disorder. *Harvard Review of Psychiatry, 2*(5), 233-245.

Anderson, E., Hamburger, S., Liu, J. H., & Rebar, R. W. (1987). Characteristics of menopausal women seeking assistance. *Am J Obstet Gynecol, 156*(2), 428-433.

APA. (1994). *DSM-IV: Diagnostic & Statistical Manual of Mental Disorders* (4th ed.). Washington, DC: American Psychiatric Press.

Appleby, L., Warner, R., Whitton, A., & Faragher, B. (1997). A controlled study of fluoxetine and cognitive-behavioural counselling in the treatment of postnatal depression. *Bmj, 314*(7085), 932-936.

Arango, V., Ernsberger, P., Marzuk, P. M., Chen, J. S., Tierney, H., Stanley, M., Reis, D. J., & Mann, J. J. (1990). Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry, 47*(11), 1038-1047.

Arora, R. C., & Meltzer, H. Y. (1988). Effect of desipramine treatment on 3H-imipramine binding in the blood platelets of depressed patients. *Biol Psychiatry, 23*(4), 397-404.

Arora, R. C., & Meltzer, H. Y. (1989). Serotonergic measures in the brains of suicide victims: 5-HT2 binding sites in the frontal cortex of suicide victims and control subjects. *Am J Psychiatry, 146*(6), 730-736.

Ashby, C. R., Jr., Carr, L. A., Cook, C. L., Steptoe, M. M., & Franks, D. D. (1988). Alteration of platelet serotonergic mechanisms and monoamine oxidase activity in premenstrual syndrome. *Biol Psychiatry, 24*(2), 225-233.

Attali, G., Weizman, A., Gil-Ad, I., & Rehavi, M. (1997). Opposite modulatory effects of ovarian hormones on rat brain dopamine and serotonin transporters. *Brain Res, 756*(1-2), 153-159.

Aylward, M. (1973). Plasma tryptophan levels and mental depression in postemnopausal subjects: Effects oforalpiperazine-oestrone sulfate. *IRCS Med Sci, 1*, 30-34.

Aylward, M. (1976). Estrogens, plasma tryptophan levels in perimenopausal patients. In S. Campbell (Ed.), *The Management of the Menopause and the Postmenopausal Years* (pp. 135-147). Lancaster: MTP Press.

Aylward, M., & Maddock, J. (1973). Total and free-plasma tryptophan concentrations in rheumatoid disease. *J Pharm Pharmacol, 25*(7), 570-572.

Baischer, W., Koinig, G., Hartmann, B., Huber, J., & Langer, G. (1995). Hypothalamic-pituitary-gonadal axis in depressed premenopausal women: elevated blood testosterone concentrations compared to normal controls. *Psychoneuroendocrinology, 20*(5), 553-559.

Baker, A., Simpson, S., & Dawson, D. (1997). Sleep disruption and mood changes associated with menopause. *J Psychosom Res, 43*(4), 359-369.

Bancroft, J., Cook, A., Davidson, D., Bennie, J., & Goodwin, G. (1991). Blunting of neuroendocrine responses to infusion of L-tryptophan in women with perimenstrual mood change. *Psychol Med, 21*(2), 305-312.

Beck, A. T., & Steer, R. A. (1987). *Manual for the Beck Depression Inventory*. San Antonio, TX: The Psychological Corporation.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J. E., & Erbaugh, J. K. (1961). An inventory for measuring depression. *Archives of General Psychiatry, 4*, 561-571.

Bel, N., & Artigas, F. (1996). Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. *J Neurochem, 67*(2), 669-676.

Bender, D. A., Laing, A. E., Vale, J. A., Papadaki, L., & Pugh, M. (1983). The effects of oestrogen administration on tryptophan metabolism in rats and in menopausal women receiving hormone replacement therapy. *Biochem Pharmacol, 32*(5), 843-848.

Benkelfat, C., Ellenbogen, M. A., Dean, P., Palmour, R. M., & Young, S. N. (1994). Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry, 51*(9), 687-697.

Bethea, C. L., Mirkes, S. J., Shively, C. A., & Adams, M. R. (2000). Steroid regulation of tryptophan hydroxylase protein in the dorsal raphe of macaques. *Biol Psychiatry, 47*(6), 562-576.

Bethea, C. L., Pecins-Thompson, M., Schutzer, W. E., Gundlah, C., & Lu, Z. N. (1998). Ovarian steroids and serotonin neural function. *Mol Neurobiol, 18*(2), 87-123.

Biggio, G., Fadda, F., Fanni, P., Tagliamonte, A., & Gessa, G. L. (1974). Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindoleacetic acid by a tryptophan-free diet. *Life Sci, 14*(7), 1321-1329.

Biver, F., Wikler, D., Lotstra, F., Damhaut, P., Goldman, S., & Mendlewicz, J. (1997). Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. *Br J Psychiatry, 171*, 444-448.

Blatt, M. G. F., Wiesbader, Y., & Kupperman, H. S. (1953). Vitamin E and climacteric syndome. *Archives of Internal Medicine, 91*, 792-795.

Bloch, M., Schmidt, P. J., & Rubinow, D. R. (1997). Premenstrual syndrome: evidence for symptom stability across cycles. *Am J Psychiatry, 154*(12), 1741-1746.

Blum, I., Vered, Y., Lifshitz, A., Harel, D., Blum, M., Nordenberg, Y., Harsat, A., Sulkes, J., Gabbay, U., & Graff, E. (1996). The effect of estrogen replacement therapy on plasma serotonin and catecholamines of postmenopausal women. *Isr J Med Sci, 32*(12), 1158-1162.

Bond, A., & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology, 47*, 211-218.

Bonnar, J., Franklin, M., Nott, P. N., & McNeilly, A. S. (1975). Effect of breast-feeding on pituitary-ovarian function after childbirth. *Br Med J, 4*(5988), 82-84.

Bowen, D. J., & Grunberg, N. E. (1990). Variations in food preference and consumption across the menstrual cycle. *Physiol Behav, 47*(2), 287-291.

Brown, C. S., Ling, F. W., Andersen, R. N., Farmer, R. G., & Arheart, K. L. (1994). Efficacy of depot leuprolide in premenstrual syndrome: effect of symptom severity and type in a controlled trial. *Obstet Gynecol, 84*(5), 779-786.

Buchanan, C. M., Eccles, J. S., & Becker, J. B. (1992). Are adolescents the victims of raging hormones: evidence for activational effects of hormones on moods and behavior at adolescence. *Psychol Bull, 111*(1), 62-107.

Buesching, D. P., Glasser, M. L., & Frate, D. A. (1986). Progression of depression in the prenatal and postpartum periods. *Women Health, 11*(2), 61-78.

Bungay, G. T., Vessey, M. P., & McPherson, C. K. (1980). Study of symptoms in middle life with special reference to the menopause. *Br Med J, 281*(6234), 181-183.

Campbell, E. M., Peterkin, D., O'Grady, K., & Sanson-Fisher, R. (1997). Premenstrual symptoms in general practice patients. Prevalence and treatment. *J Reprod Med, 42*(10), 637-646.

Campbell, J. L. (1992). Maternity blues: A Model for biological research. In P. N. Harberger (Ed.), *Postpartum Psychiatric Illness: A Picture Puzzle* (pp. 90-101). Philadelphia: University of Pennsylvania Press.

Campbell, S. (1976). Double blind psychometric studies on the effects of natural estrogens on post-menopausal women, *The Management of the Menopausal and Post-Menopausal Years* (pp. 159-168). Baltimore: University Park Press.

Carpenter, L. L., Anderson, G. M., Pelton, G. H., Gudin, J. A., Kirwin, P. D., Price, L. H., Heninger, G. R., & McDougle, C. J. (1998). Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology, 19*(1), 26-35.

Carr, B. R., & MacDonald, P. C. (1983). Estrogen treatment of postmenopausal women. *Adv Intern Med, 28*, 491-508.

Carranza-Lira, S., & Valentino-Figueroa, M. L. (1999). Estrogen therapy for depression in postmenopausal women. *Int J Gynaecol Obstet, 65*(1), 35-38.

Casper, R. F., & Hearn, M. T. (1990). The effect of hysterectomy and bilateral oophorectomy in women with severe premenstrual syndrome. *Am J Obstet Gynecol, 162*(1), 105-109.

Charney, D. S., Heninger, G. R., Reinhard, J. F., Jr., Sternberg, D. E., & Hafstead, K. M. (1982). The effect of intravenous L-tryptophan on prolactin and growth hormone and mood in healthy subjects. *Psychopharmacology, 77*(3), 217-222.

Chompootweep, S., Tankeyoon, M., Yamarat, K., Poomsuwan, P., & Dusitsin, N. (1993). The menopausal age and climacteric complaints in Thai women in Bangkok. *Maturitas, 17*(1), 63-71.

Christensen, L., & Redig, C. (1993). Effect of meal composition on mood. *Behav Neurosci, 107*(2), 346-353.

Cleare, A. J., Murray, R. M., & O'Keane, V. (1996). Reduced prolactin and cortisol responses to d-fenfluramine in depressed compared to healthy matched control subjects. *Neuropsychopharmacology, 14*(5), 349-354.

Coppen, A., Brooksbank, B. W., & Peet, M. (1972). Tryptophan concentration in the cerebrospinal fluid of depressive patients. *Lancet, 1*(7765), 1393.

Coppen, A., Eccleston, E. G., & Peet, M. (1973). Total and free tryptophan concentration in the plasma of depressive patients. *Lancet, 2*(7820), 60-63.

Coppen, A., & Wood, K. (1978). Tryptophan and depressive illness. *Psychol Med, 8*(1), 49-57.

Cowen, P. J., & Charig, E. M. (1987). Neuroendocrine responses to intravenous tryptophan in major depression. *Arch Gen Psychiatry, 44*(11), 958-966.

Cowen, P. J., Parry-Billings, M., & Newsholme, E. A. (1989). Decreased plasma tryptophan levels in major depression. *J Affect Disord, 16*(1), 27-31.

Cox, J. L., Murray, D., & Chapman, G. (1993). A controlled study of the onset, duration and prevalence of postnatal depression. *Br J Psychiatry, 163*, 27-31.

Cyr, M., Bosse, R., & Di Paolo, T. (1998). Gonadal hormones modulate 5-hydroxytryptamine2A receptors: emphasis on the rat frontal cortex. *Neuroscience, 83*(3), 829-836.

Da-Silva, V. A., Moraes-Santos, A. R., Carvalho, M. S., Martins, M. L., & Teixeira, N. A. (1998). Prenatal and postnatal depression among low income Brazilian women. *Braz J Med Biol Res, 31*(6), 799-804.

Deakin, J. F. W. (1988). Relevance of hormone-CNS interactions to psychological changes in the puerperium. In I. F. Brockington (Ed.), *Motherhood and Mental Illness 2: Causes and Consequences* (pp. 113-132). London: Butterworth.

DeCherney, A. (1993). Physiologic and pharmacologic effects of estrogen and progestins on bone. *J Reprod Med, 38*(12 Suppl), 1007-1014.

Delgado, P. L., Charney, D. S., Price, L. H., Aghajanian, G. K., Landis, H., & Heninger, G. R. (1990). Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry, 47*(5), 411-418.

Delgado, P. L., Miller, H. L., Salomon, R. M., Licinio, J., Krystal, J. H., Moreno, F. A., Heninger, G. R., & Charney, D. S. (1999). Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry, 46*(2), 212-220.

Delgado, P. L., Price, L. H., Miller, H. L., Salomon, R. M., Aghajanian, G. K., Heninger, G. R., & Charney, D. S. (1994). Serotonin and the neurobiology of depression. Effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatry, 51*(11), 865-874.

DeMyer, M. K., Shea, P. A., Hendrie, H. C., & Yoshimura, N. N. (1981). Plasma tryptophan and five other amino acids in depressed and normal subjects. *Arch Gen Psychiatry, 38*(6), 642-646.

Dennerstein, L., Brown, J. B., Gotts, G., Morse, C. A., Farley, T. M., & Pinol, A. (1993). Menstrual cycle hormonal profiles of women with and without premenstrual syndrome. *J Psychosom Obstet Gynaecol, 14*(4), 259-268.

Dennerstein, L., Burrows, G. D., Hyman, G. J., & Sharpe, K. (1979). Hormone therapy and affect. *Maturitas, 1*(4), 247-259.

Dennerstein, L., Morse, C., & Gotts, G. (1988). Perspective from a PMS clinic. In R. L. Berkowitz (Ed.), *The premenstrual syndroms* (pp. 109-118). New York: Churchill Livingstone.

Dinan, T. G., Barry, S., Yatham, L. N., Mobayed, M., & O'Hanlon, M. (1990). The reproducibility of the prolactin response to buspirone: relationship to the menstrual cycle. *Int Clin Psychopharmacol, 5*(2), 119-123.

Ditkoff, E. C., Crary, W. G., Cristo, M., & Lobo, R. A. (1991). Estrogen improves psychological function in asymptomatic postmenopausal women. *Obstet Gynecol, 78*(6), 991-995.

Drevets, W. C., Frank, E., Price, J. C., Kupfer, D. J., Holt, D., Greer, P. J., Huang, Y., Gautier, C., & Mathis, C. (1999). PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry, 46*(10), 1375-1387.

Eich, E., Macaulay, D., & Ryan, L. (1994). Mood dependent memory for events of the personal past. *J Exp Psychol Gen, 123*(2), 201-215.

Ellenbogen, M. A., Young, S. N., Dean, P., Palmour, R. M., & Benkelfat, C. (1996). Mood response to acute tryptophan depletion in healthy volunteers: sex differences and temporal stability. *Neuropsychopharmacology, 15*(5), 465-474.

Ellenbogen, M. A., Young, S. N., Dean, P., Palmour, R. M., & Benkelfat, C. (1999). Acute tryptophan depletion in healthy young women with a family history of major affective disorder. *Psychol Med, 29*(1), 35-46.

Feldman, R. S., Meyer, J. S., & Quenzer, L. F. (1997). *Principles of Neuropsychopharmacology*. Sunderland: Sinauer Associates.

Fernstrom, J. D., & Faller, D. V. (1978). Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem, 30*(6), 1531-1538.

Fernstrom, J. D., & Wurtman, R. J. (1971). Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science, 173*(992), 149-152.

Fernstrom, J. D., & Wurtman, R. J. (1972). Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science, 178*(59), 414-416.

Fink, G., & Sumner, B. E. (1996). Oestrogen and mental state. *Nature, 383*(6598), 306.

Fink, G., Sumner, B. E., Rosie, R., Grace, O., & Quinn, J. P. (1996). Estrogen control of central neurotransmission: effect on mood, mental state, and memory. *Cell Mol Neurobiol, 16*(3), 325-344.

First, M. B., Williams, J. B., Gibbon, M., & Spitzer, R. L. (1997). *Structured Clinical Interview for DSM-IV AXIS I Disorders (SCID-I)*. Washington, DC: American Psychiatric Press.

FitzGerald, M., Malone, K. M., Li, S., Harrison, W. M., McBride, P. A., Endicott, J., Cooper, T., & Mann, J. J. (1997). Blunted serotonin response to fenfluramine challenge in premenstrual dysphoric disorder. *Am J Psychiatry, 154*(4), 556-558.

Frank, R. T. (1931). The hormonal causes of premenstrual tension. *Arch Neurol Psychiatry, 26*, 1053-1057.

Freeman, E. W., Rickels, K., Sondheimer, S. J., & Polansky, M. (1999). Differential response to antidepressants in women with premenstrual syndrome/premenstrual dysphoric disorder: a randomized controlled trial. *Arch Gen Psychiatry, 56*(10), 932-939.

Freeman, E. W., Sondheimer, S. J., & Rickels, K. (1997). Gonadotropin-releasing hormone agonist in the treatment of premenstrual symptoms with and without ongoing dysphoria: a controlled study. *Psychopharmacol Bull, 33*(2), 303-309.

Gallagher, D. (1986). The beck depression inventory and older adults: Review of its development and utility. *Clinical Gerontologist, 5*(1/2), 149-163.

Gard, P. R., Handley, S. L., Parsons, A. D., & Waldron, G. (1986). A multivariate investigation of postpartum mood disturbance. *Br J Psychiatry, 148*, 567-575.

Gater, R., Tansella, M., Korten, A., Tiemens, B. G., Mavreas, V. G., & Olatawura, M. O. (1998). Sex differences in the prevalence and detection of depressive and anxiety disorders in general health care settings: report from the World Health Organization Collaborative Study on Psychological Problems in General Health Care. *Arch Gen Psychiatry, 55*(5), 405-413.

Georgiopoulos, A. M., Bryan, B. P., Yawn, B. P., Houston, M. S., Rummans, T. A., & Therneau, T. M. (1999). Population-based screening for postpartum depression. *Obstetrics and Gynecology, 93*(5), 653-657.

Gessa, G. L., Biggio, G., Fadda, F., Corsini, G. U., & Tagliamonte, A. (1974). Effect of the oral administration of tryptophan-free amino acid mixtures on serum tryptophan, brain tryptophan and serotonin metabolism. *J Neurochem, 22*(5), 869-870.

Gonzales, G. F., & Carrillo, C. (1993). Blood serotonin levels in postmenopausal women: effects of age and serum oestradiol levels. *Maturitas, 17*(1), 23-29.

Goonaratna, C., Fonseka, P., & Wijeywardene, K. (1999). Perimenopausal symptoms in Sri Lankan women. *Ceylon Med J, 44*(2), 63-69.

Greendale, G. A., Reboussin, B. A., Hogan, P., Barnabei, V. M., Shumaker, S., Johnson, S., & Barrett-Connor, E. (1998). Symptom relief and side effects of postmenopausal hormones: results from the Postmenopausal Estrogen/Progestin Interventions Trial. *Obstet Gynecol, 92*(6), 982-988.

Gregoire, A. J., Kumar, R., Everitt, B., Henderson, A. F., & Studd, J. W. (1996). Transdermal oestrogen for treatment of severe postnatal depression. *Lancet, 347*(9006), 930-933.

Halbreich, U. (2000). Gonadal hormones, reproductive age, and women with depression. *Arch Gen Psychiatry, 57*(12), 1163-1164.

Halbreich, U., & Endicott, J. (1985). Relationship of dysphoric premenstrual changes to depressive disorders. *Acta Psychiatr Scand, 71*(4), 331-338.

Halbreich, U., Endicott, J., Goldstein, S., & Nee, J. (1986). Premenstrual changes and changes in gonadal hormones. *Acta Psychiatr Scand, 74*(6), 576-586.

Halbreich, U., Rojansky, N., Palter, S., Tworek, H., Hissin, P., & Wang, K. (1995). Estrogen augments serotonergic activity in postmenopausal women. *Biol Psychiatry, 37*(7), 434-441.

Hamilton, J. A. (1989). Postpartum psychiatric syndromes. *Psychiatr Clin North Am, 12*(1), 89-103.

Hamilton, J. A., Parry, B. L., & Blumenthal, S. J. (1988). The menstrual cycle in context, II: Human gonadal steroid hormone variability. *J Clin Psychiatry, 49*(12), 480-484.

Hammarback, S., Damber, J. E., & Backstrom, T. (1989). Relationship between symptom severity and hormone changes in women with premenstrual syndrome. *J Clin Endocrinol Metab, 68*(1), 125-130.

Handley, S. L., Dunn, T. L., Baker, J. M., Cockshott, C., & Gould, S. (1977). Mood changes in puerperium, and plasma tryptophan and cortisol concentrations. *Br Med J, 2*(6078), 18-20.

Handley, S. L., Dunn, T. L., Waldron, G., & Baker, J. M. (1980). Tryptophan, cortisol and puerperal mood. *Br J Psychiatry, 136*, 498-508.

Hannah, P., Adams, D., Glover, V., & Sandler, M. (1992). Abnormal platelet 5-hydroxytryptamine uptake and imipramine binding in postnatal dysphoria. *J Psychiatr Res, 26*(1), 69-75.

Harris, B. (1980). Prospective trial of L-tryptophan in maternity blues. *Br J Psychiatry, 137*, 233-235.

Harrison, W. M., Endicott, J., Rabkin, J. G., & Nee, J. (1984). Treatment of premenstrual dysphoric changes: clinical outcome and methodological implications. *Psychopharmacol Bull, 20*(1), 118-122.

Hearn, G., Iliff, A., Jones, I., Kirby, A., Ormiston, P., Parr, P., Rout, J., & Wardman, L. (1998). Postnatal depression in the community. *Br J Gen Pract, 48*(428), 1064-1066.

Heidrich, A., Schleyer, M., Spingler, H., Albert, P., Knoche, M., Fritze, J., & Lanczik, M. (1994). Postpartum blues: relationship between not-protein bound steroid hormones in plasma and postpartum mood changes. *J Affect Disord, 30*(2), 93-98.

Heninger, G. R., Charney, D. S., & Sternberg, D. E. (1984). Serotonergic function in depression. Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. *Arch Gen Psychiatry, 41*(4), 398-402.

Hrboticky, N., Leiter, L. A., & Anderson, G. H. (1989). Menstrual cycle effects on the metabolism of tryptophan loads. *Am J Clin Nutr, 50*(1), 46-52.

Hylan, T. R., Sundell, K., & Judge, R. (1999). The impact of premenstrual symptomatology on functioning and treatment-seeking behavior: experience from the United States, United Kingdom, and France. *J Womens Health Gend Based Med, 8*(8), 1043-1052.

Jacobs, B. L. (1991). Serotonin and behavior: emphasis on motor control. *J Clin Psychiatry, 52 Suppl*, 17-23.

Jacobs, B. L., & Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiol Rev, 72*(1), 165-229.

Jacobs, B. L., & Fornal, C. A. (1997). Serotonin and motor activity. *Curr Opin Neurobiol, 7*(6), 820-825.

Johnson, S. R., McChesney, C., & Bean, J. A. (1988). Epidemiology of premenstrual symptoms in a nonclinical sample. I. Prevalence, natural history and help-seeking behavior. *J Reprod Med, 33*(4), 340-346.

Kendall, D. A., Stancel, G. M., & Enna, S. J. (1981). Imipramine: effect of ovarian steroids on modifications in serotonin receptor binding. *Science, 211*(4487), 1183-1185.

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *Am J Psychiatry, 158*(4), 582-586.

Kenealy, P. M. (1997). Mood-state-dependent retrieval: the effects of induced mood on memory reconsidered. *Q J Exp Psychol A, 50*(2), 290-317.

Kessler, R. C., McGonagle, K. A., Swartz, M., Blazer, D. G., & Nelson, C. B. (1993). Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord, 29*(2-3), 85-96.

Klaassen, T., Riedel, W. J., Deutz, N. E., van Someren, A., & van Praag, H. M. (1999). Specificity of the tryptophan depletion method. *Psychopharmacology (Berl), 141*(3), 279-286.

Klaassen, T., Riedel, W. J., van Someren, A., Deutz, N. E., Honig, A., & van Praag, H. M. (1999). Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiatry, 46*(4), 489-497.

Klaiber, E., Broverman, D., Vogel, W., & Kobayashi, M. (1974). Rhythms in plasma MAO activity, EEG and behavior during tthe menstrual cycle. In R. L. V. d. Wiele (Ed.), *Biorhythms and Human Reproduction* (pp. 124-155). New York: John Wiley and Sons.

Klaiber, E. L., Broverman, D. M., Vogel, W., & Kobayashi, Y. (1979). Estrogen therapy for severe persistent depressions in women. *Arch Gen Psychiatry, 36*(5), 550-554.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., & Gustafsson, J. A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A, 93*(12), 5925-5930.

Lam, R. W., Zis, A. P., Grewal, A., Delgado, P. L., Charney, D. S., & Krystal, J. H. (1996). Effects of rapid tryptophan depletion in patients with seasonal affective disorder in remission after light therapy. *Arch Gen Psychiatry, 53*(1), 41-44.

Leyton, M., Young, S. N., Blier, P., Ellenbogen, M. A., Palmour, R. M., Ghadirian, A. M., & Benkelfat, C. (1997). The effect of tryptophan depletion on mood in medication-free, former patients with major affective disorder. *Neuropsychopharmacology, 16*(4), 294-297.

Leyton, M., Young, S. N., Pihl, R. O., Etezadi, S., Lauze, C., Blier, P., Baker, G. B., & Benkelfat, C. (2000). Effects on mood of acute phenylalanine/tyrosine depletion in healthy women. *Neuropsychopharmacology, 22*(1), 52-63.

Lippert, T. H., Filshie, M., Muck, A. O., Seeger, H., & Zwirner, M. (1996). Serotonin metabolite excretion after postmenopausal estradiol therapy. *Maturitas, 24*(1-2), 37-41.

Longcope, C. (1990). Hormone dynamics at the menopause. *Ann N Y Acad Sci, 592*, 21-30.

Lorr, M., & McNair, D. (1982). *Profile of mood states: Bi-polar form (POMS-BI)*. San Diego, CA: Educational and Industrial Testing Service.

Lucca, A., Lucini, V., Catalano, M., Alfano, M., & Smeraldi, E. (1994). Plasma tryptophan to large neutral amino acids ratio and therapeutic response to a selective serotonin uptake inhibitor. *Neuropsychobiology, 29*(3), 108-111.

Luine, V. N., Khylchevskaya, R. I., & McEwen, B. S. (1975). Effect of gonadal steroids on activities of monoamine oxidase and choline acetylase in rat brain. *Brain Res, 86*(2), 293-306.

Luine, V. N., & McEwen, B. S. (1977). Effect of oestradiol on turnover of type A monoamine oxidase in brain. *J Neurochem, 28*(6), 1221-1227.

Maes, M., Claes, M., Schotte, C., Delbeke, L., Jacquemyn, Y., Verkerk, R., De Meester, I., & Scharpe, S. (1992). Disturbances in dexamethasone suppression test and lower availability of L-tryptophan and tyrosine in early puerperium and in women under contraceptive therapy. *J Psychosom Res, 36*(2), 191-197.

Maes, M., & Meltzer, H. Y. (1995). The serotonin hypothesis of major depression. In D. J. Kupfer (Ed.), *Psychopharmacology: The Fourth Generation of Progress* (pp. 933-944). New York: Raven Press.

Malison, R. T., Price, L. H., Berman, R., van Dyck, C. H., Pelton, G. H., Carpenter, L., Sanacora, G., Owens, M. J., Nemeroff, C. B., Rajeevan, N., Baldwin, R. M., Seibyl, J. P., Innis, R. B., & Charney, D. S. (1998). Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. *Biol Psychiatry, 44*(11), 1090-1098.

Maloney, D. M. (1998). Postnatal depression: a study of mothers in the metropolitan area of Perth, Western Australia. *J Aust Coll Midwives, 11*(2), 18-23.

Mann, J. J. (1998). The neurobiology of suicide. *Nat Med, 4*(1), 25-30.

Mann, J. J. (1999). Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology, 21*(2 Suppl), 99S-105S.

Mann, J. J., Malone, K. M., Diehl, D. J., Perel, J., Cooper, T. B., & Mintun, M. A. (1996). Demonstration in vivo of reduced serotonin responsivity in the brain of untreated depressed patients. *Am J Psychiatry, 153*(2), 174-182.

Mann, J. J., McBride, P. A., Malone, K. M., DeMeo, M., & Keilp, J. (1995). Blunted serotonergic responsivity in depressed inpatients. *Neuropsychopharmacology, 13*(1), 53-64.

McEwen, B. S. (1994). Ovarian Steroids have diverse effects on brain structure and function. In M. Hammar (Ed.), *The Modern Management of the Menopause: A perspective for the 21st Century* (pp. 269-278). New York: Parthenon Publishing Group.

McEwen, B. S., & Alves, S. E. (1999). Estrogen actions in the central nervous system. *Endocr Rev, 20*(3), 279-307.

McEwen, B. S., & Parsons, B. (1982). Gonadal steroid action on the brain: neurochemistry and neuropharmacology. *Annu Rev Pharmacol Toxicol, 22*, 555-598.

McMenamy, R. H., & Onclet, J. L. (1958). Specific Binding of L-tryptophan to serum albumin. *J Biol Chem, 233*, 1436.

McQueen, J. K., Wilson, H., & Fink, G. (1997). Estradiol-17 beta increases serotonin transporter (SERT) mRNA levels and the density of SERT-binding sites in female rat brain. *Brain Res Mol Brain Res, 45*(1), 13-23.

Meltzer, H. Y., Arora, R. C., Baber, R., & Tricou, B. J. (1981). Serotonin uptake in blood platelets of psychiatric patients. *Arch Gen Psychiatry, 38*(12), 1322-1326.

Menkes, D. B., Coates, D. C., & Fawcett, J. P. (1994). Acute tryptophan depletion aggravates premenstrual syndrome. *J Affect Disord, 32*(1), 37-44.

Merikangas, K. R., Foeldenyi, M., & Angst, J. (1993). The Zurich Study. XIX. Patterns of menstrual disturbances in the community: results of the Zurich Cohort Study. *Eur Arch Psychiatry Clin Neurosci, 243*(1), 23-32.

Metcalf, M. G., & Livesey, J. H. (1995). Distribution of positive moods in women with the premenstrual syndrome and in normal women. *J Psychosom Res, 39*(5), 609-618.

Miller, M. M., & Franklin, K. B. (1999). Theoretical basis for the benefit of postmenopausal estrogen substitution. *Exp Gerontol, 34*(5), 587-604.

Moja, E. A., Restani, P., Corsini, E., Stacchezzini, M. C., Assereto, R., & Galli, C. L. (1991). Cycloheximide blocks the fall of plasma and tissue tryptophan levels after tryptophan-free amino acid mixtures. *Life Sci, 49*(15), 1121-1128.

Moller, S. E., Maach-Moller, B., Olesen, M., & Fjalland, B. (1993). Effect of oral contraceptives on precursor amino acids. In R. Murison (Ed.), *Endocrine and nutritional control of basic biological functions*. Toronto: Hogrefe and Huber.

Monagle, L., Dan, A., Krogh, V., Jossa, F., Farinaro, E., & Trevisan, M. (1993). Perimenstrual symptom prevalence rates: an Italian-American comparison. *Am J Epidemiol, 138*(12), 1070-1081.

Montgomery, J. C., Appleby, L., Brincat, M., Versi, E., Tapp, A., Fenwick, P. B., & Studd, J. W. (1987). Effect of oestrogen and testosterone implants on psychological disorders in the climacteric. *Lancet, 1*(8528), 297-299.

Moreno, F. A., Gelenberg, A. J., Heninger, G. R., Potter, R. L., McKnight, K. M., Allen, J., Phillips, A. P., & Delgado, P. L. (1999). Tryptophan depletion and depressive vulnerability. *Biol Psychiatry, 46*(4), 498-505.

Mueck, A. O., Seeger, H., Kasspohl-Butz, S., Teichmann, A. T., & Lippert, T. H. (1997). Influence of norethisterone acetate and estradiol on the serotonin metabolism of postmenopausal women. *Horm Metab Res, 29*(2), 80-83.

Munaro, N. I. (1978). The effect of ovarian steroids on hypothalamic 5-hydroxytryptamine neuronal activity. *Neuroendocrinology, 26*(5), 270-276.

Neumeister, A., Praschak-Rieder, N., Besselmann, B., Rao, M. L., Gluck, J., & Kasper, S. (1997). Effects of tryptophan depletion on drug-free patients with seasonal affective disorder during a stable response to bright light therapy. *Arch Gen Psychiatry, 54*(2), 133-138.

Nishizawa, S., Benkelfat, C., Young, S. N., Leyton, M., Mzengeza, S., de Montigny, C., Blier, P., & Diksic, M. (1997). Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A, 94*(10), 5308-5313.

Nott, P. N., Franklin, M., Armitage, C., & Gelder, M. G. (1976). Hormonal changes and mood in the puerperium. *Br J Psychiatry, 128*, 379-383.

Nurnberger, J. I., Blehar, M. C., Kaufmann, C. A., York-Cooler, C., Simpson, S. G., Harkavy-Friedman, J., Severe, J. B., Malaspina, D., Reich, T., Miller, M. , Bowman, E. S., Depaulo, J. R., Cloninger, C. R., Robinson, G., Modlin, S., Gershon, E. S., Maxwell, E., Guroff, J. J., Kirch, D., Wynne, D., Berg, K., Tsuang, M. T., Faraone, S. V., Pepple, J. R., & Ritz, A. L. (1994). Diagnositic interview for genetic studies: Rationale, unique features, and training. *Archives of General Psychiatry, 51*, 849-859.

O'Hara, M. W., Schlechte, J. A., Lewis, D. A., & Wright, E. J. (1991). Prospective study of postpartum blues. Biologic and psychosocial factors. *Arch Gen Psychiatry, 48*(9), 801-806.

O'Keane, V., & Dinan, T. G. (1991). Prolactin and cortisol responses to d-fenfluramine in major depression: evidence for diminished responsivity of central serotonergic function. *Am J Psychiatry, 148*(8), 1009-1015.

Oldendorf, W. H., & Szabo, J. (1976). Amino acid assignment to one of three blood-brain barrier amino acid carriers. *Am J Physiol, 230*(1), 94-98.

Oldman, A. D., Walsh, A. E. S., Salkovskis, P., Laver, D. A., & Cowen, P. J. (1994). Effect of acute tryptophan depletion on mood and appetitie in healthy female volunteers. *Journal of Psychopharmacology, 8*(1), 8-13.

Oppenheim, G. (1983). Estrogen in the treatment of depression: neuropharmacological mechanisms. *Biol Psychiatry, 18*(6), 721-725.

Osterlund, M. K., Gustafsson, J. A., Keller, E., & Hurd, Y. L. (2000). Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERalpha mRNA. *J Clin Endocrinol Metab, 85*(10), 3840-3846.

Paffenbarger, R. S. (1982). Epidemiological aspects of mental illness associated with childbearing. In R. Kumar (Ed.), *Motherhood and Mental Illness* (pp. 19-36). London: Academic Press.

Parry, B. L. (1992). Reproductive-related depressions in women: Phenomena of hormonal kindling? In P. N. Harberger (Ed.), *Postpartum Psychiatric Illness: A Picture Puzzle* (pp. 200-218). Philadelphia: University of Pennsylvania Press.

Parry, B. L. (1995). Mood disorders linked to the reproductive cycle in women. In D. J. Kupfer (Ed.), *Psychopharmacology: The Fourth Generation of Progress* (pp. 1029-1042). New York: Raven Press.

Paul, S. M., Rehavi, M., Skolnick, P., Ballenger, J. C., & Goodwin, F. K. (1981). Depressed patients have decreased binding of tritiated imipramine to platelet serotonin "transporter". *Arch Gen Psychiatry, 38*(12), 1315-1317.

Pearlstein, T., Rosen, K., & Stone, A. B. (1997). Mood disorders and menopause. *Endocrinol Metab Clin North Am, 26*(2), 279-294.

Pecins-Thompson, M., & Bethea, C. L. (1999). Ovarian steroid regulation of serotonin-1A autoreceptor messenger RNA expression in the dorsal raphe of rhesus macaques. *Neuroscience, 89*(1), 267-277.

Pecins-Thompson, M., Brown, N. A., & Bethea, C. L. (1998). Regulation of serotonin re-uptake transporter mRNA expression by ovarian steroids in rhesus macaques. *Brain Res Mol Brain Res, 53*(1-2), 120-129.

Pecins-Thompson, M., Brown, N. A., Kohama, S. G., & Bethea, C. L. (1996). Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in rhesus macaques. *J Neurosci, 16*(21), 7021-7029.

Pop, V. J., Essed, G. G., de Geus, C. A., van Son, M. M., & Komproe, I. H. (1993). Prevalence of post partum depression--or is it post-puerperium depression? *Acta Obstet Gynecol Scand, 72*(5), 354-358.

Post, R. M. (1992). Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. *Am J Psychiatry, 149*(8), 999-1010.

Post, R. M., & Weiss, S. R. (1992). Ziskind-Somerfeld Research Award 1992. Endogenous biochemical abnormalities in affective illness: therapeutic versus pathogenic. *Biol Psychiatry, 32*(6), 469-484.

Price, L. H., Charney, D. S., Delgado, P. L., & Heninger, G. R. (1991). Serotonin function and depression: neuroendocrine and mood responses to intravenous L-tryptophan in depressed patients and healthy comparison subjects. *Am J Psychiatry, 148*(11), 1518-1525.

Raleigh, M. J., Brammer, G. L., Yuwiler, J. W., Flannery, J. W., McGuire, M. T., & Geller, E. (1980). Serotonergic influences in the social behavior of vervet monkeys. *Experimental Neurology, 68*, 322-334.

Rapkin, A., Chung, L. C., & Reading, A. (1989). tryptophan loading test in premenstrual syndrome. *Journal of Obstetrics and Gynecology, 10*, 140-144.

Rapkin, A. J., Chang, L. C., & Reading, A. E. (1989). Mood and cognitive style in premenstrual syndrome. *Obstet Gynecol, 74*(4), 644-649.

Rapkin, A. J., Reading, A. E., Woo, S., & Goldman, L. M. (1991). Tryptophan and neutral amino acids in premenstrual syndrome. *Am J Obstet Gynecol, 165*(6 Pt 1), 1830-1833.

Rasgon, N., McGuire, M., Tanavoli, S., Fairbanks, L., & Rapkin, A. (2000). Neuroendocrine response to an intravenous L-tryptophan challenge in women with premenstrual syndrome. *Fertil Steril, 73*(1), 144-149.

Redei, E., & Freeman, E. W. (1995). Daily plasma estradiol and progesterone levels over the menstrual cycle and their relation to premenstrual symptoms. *Psychoneuroendocrinology, 20*(3), 259-267.

Rickels, K., Freeman, E., & Sondheimer, S. (1989). Buspirone in treatment of premenstrual syndrome. *Lancet, 1*(8641), 777.

Rivera-Tovar, A. D., & Frank, E. (1990). Late luteal phase dysphoric disorder in young women. *Am J Psychiatry, 147*(12), 1634-1636.

Rubinow, D. R., & Schmidt, P. J. (1995). The treatment of premenstrual syndrome--forward into the past. *N Engl J Med, 332*(23), 1574-1575.

Russ, M. J., Ackerman, S. H., Banay-Schwartz, M., Shindledecker, R. D., & Smith, G. P. (1990). Plasma tryptophan to large neutral amino acid ratios in depressed and normal subjects. *J Affect Disord, 19*(1), 9-14.

Sagsoz, N., Oguzturk, O., Bayram, M., & Kamaci, M. (2001). Anxiety and depression before and after the menopause. *Arch Gynecol Obstet, 264*(4), 199-202.

Sargent, P. A., Kjaer, K. H., Bench, C. J., Rabiner, E. A., Messa, C., Meyer, J., Gunn, R. N., Grasby, P. M., & Cowen, P. J. (2000). Brain serotonin1A receptor binding

measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry, 57*(2), 174-180.

Sator, M. O., Wieser, F., Gruber, D. M., Joura, E. A., & Huber, J. C. (1999). Trends in the prescription of psychotropic drugs and hormone substitutes in Austria. *Wien Klin Wochenschr, 111*(10), 402-405.

Schmidt, P. J., Nieman, L., Danaceau, M. A., Tobin, M. B., Roca, C. A., Murphy, J. H., & Rubinow, D. R. (2000). Estrogen replacement in perimenopausal-related depression: A preliminary report. *American Journal of Obstetrics and Gynecology, 183*(2), 414-420.

Schmidt, P. J., Nieman, L. K., Danaceau, M. A., Adams, L. F., & Rubinow, D. R. (1998). Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. *N Engl J Med, 338*(4), 209-216.

Schneider, L. S., Small, G. W., Hamilton, S. H., Bystritsky, A., Nemeroff, C. B., & Meyers, B. S. (1997). Estrogen replacement and response to fluoxetine in a multicenter geriatric depression trial. Fluoxetine Collaborative Study Group. *Am J Geriatr Psychiatry, 5*(2), 97-106.

Seeman, M. V. (1997). Psychopathology in women and men: focus on female hormones. *Am J Psychiatry, 154*(12), 1641-1647.

Sherwin, B. B. (1988). Affective changes with estrogen and androgen replacement therapy in surgically menopausal women. *J Affect Disord, 14*(2), 177-187.

Sherwin, B. B., & Gelfand, M. M. (1985). Sex steroids and affect in the surgical menopause: a double-blind, cross-over study. *Psychoneuroendocrinology, 10*(3), 325-335.

Sherwin, B. B., & Suranyi-Cadotte, B. E. (1990). Up-regulatory effect of estrogen on platelet 3H-imipramine binding sites in surgically menopausal women. *Biol Psychiatry, 28*(4), 339-348.

Shimizu, H., & Bray, G. A. (1993). Effects of castration, estrogen replacement and estrus cycle on monoamine metabolism in the nucleus accumbens, measured by microdialysis. *Brain Res, 621*(2), 200-206.

Shopsin, B., Friedman, E., & Gershon, S. (1976). Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. *Arch Gen Psychiatry, 33*(7), 811-819.

Shopsin, B., Gershon, S., Goldstein, M., Friedman, E., & Wilk, S. (1975). Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. *Psychopharmacol Commun, 1*(2), 239-249.

Sichel, D. A., Cohen, L. S., Robertson, L. M., Ruttenberg, A., & Rosenbaum, J. F. (1995). Prophylactic estrogen in recurrent postpartum affective disorder. *Biol Psychiatry, 38*(12), 814-818.

Singh, B. B., Berman, B. M., Simpson, R. L., & Annechild, A. (1998). Incidence of premenstrual syndrome and remedy usage: a national probability sample study. *Altern Ther Health Med, 4*(3), 75-79.

Sitruk-Ware, R. (1995). Cardiovascular risk at the menopause--role of sexual steroids. *Horm Res, 43*(1-3), 58-63.

Skegg, D. C., Doll, R., & Perry, J. (1977). Use of medicines in general practice. *Br Med J, 1*(6076), 1561-1563.

Smith, K. A., Fairburn, C. G., & Cowen, P. J. (1997). Relapse of depression after rapid depletion of tryptophan. *Lancet, 349*(9056), 915-919.

Smith, K. A., Fairburn, C. G., & Cowen, P. J. (1999). Symptomatic relapse in bulimia nervosa following acute tryptophan depletion. *Arch Gen Psychiatry, 56*(2), 171-176.

Smith, R. N., Studd, J. W., Zamblera, D., & Holland, E. F. (1995). A randomised comparison over 8 months of 100 micrograms and 200 micrograms twice weekly doses of transdermal oestradiol in the treatment of severe premenstrual syndrome. *Br J Obstet Gynaecol, 102*(6), 475-484.

Soares, C. N., Almeida, O. P., Joffe, H., & Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch Gen Psychiatry, 58*(6), 529-534.

Spillmann, M. K., Van der Does, A. J., Rankin, M. A., Vuolo, R. D., Alpert, J. E., Nierenberg, A. A., Rosenbaum, J. F., Hayden, D., Schoenfeld, D., & Fava, M. (2001). Tryptophan depletion in SSRI-recovered depressed outpatients. *Psychopharmacology (Berl), 155*(2), 123-127.

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord, 51*(3), 215-235.

Stancampiano, R., Melis, F., Sarais, L., Cocco, S., Cugusi, C., & Fadda, F. (1997). Acute administration of a tryptophan-free amino acid mixture decreases 5-HT release in rat hippocampus in vivo. *Am J Physiol, 272*(3 Pt 2), R991-994.

Stanley, M., & Mann, J. J. (1983). Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet, 1*(8318), 214-216.

Stein, G., Milton, F., Bebbington, P., Wood, K., & Coppen, A. (1976). Relationship between mood disturbances and free and total plasma tryptophan in postpartum women. *British Medical Journal, 2*, 457.

Steinberg, S. (1991). The treatment of late luteal phase dysphoric disorder. *Life Sci, 49*(11), 767-802.

Steinberg, S., Annable, L., Young, S. N., & Liyanage, N. (1999). A placebo-controlled study of the effects of L-tryptophan in patients with premenstrual dysphoria. *Adv Exp Med Biol, 467*, 85-88.

Steiner, M. (1979). Psychobiology of mental disorders associated with childbearing. An overview. *Acta Psychiatr Scand, 60*(5), 449-464.

Steiner, M. (1997). Premenstrual syndromes. *Annu Rev Med, 48*, 447-455.

Steiner, M., Steinberg, S., Stewart, D., Carter, D., Berger, C., Reid, R., Grover, D., & Streiner, D. (1995). Fluoxetine in the treatment of premenstrual dysphoria. Canadian Fluoxetine/Premenstrual Dysphoria Collaborative Study Group. *N Engl J Med, 332*(23), 1529-1534.

Stephens, T., Dulberg, C., & Joubert, N. (1999). Mental health of the Canadian population: a comprehensive analysis. *Chronic Dis Can, 20*(3), 118-126.

Su, T. P., Schmidt, P. J., Danaceau, M. A., Tobin, M. B., Rosenstein, D. L., Murphy, D. L., & Rubinow, D. R. (1997). Fluoxetine in the treatment of premenstrual dysphoria. *Neuropsychopharmacology, 16*(5), 346-356.

Summer, B. E., & Fink, G. (1995). Estrogen increases the density of 5-hydroxytryptamine(2A) receptors in cerebral cortex and nucleus accumbens in the female rat. *J Steroid Biochem Mol Biol, 54*(1-2), 15-20.

Sumner, B. E. H., & Fink, G. (1993). Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. *Mol. Cell. Neurosci., 4*(1), 83-92.

Taylor, D. L., Mathew, R. J., Ho, B. T., & Weinman, M. L. (1984). Serotonin levels and platelet uptake during premenstrual tension. *Neuropsychobiology, 12*(1), 16-18.

Thomson, J., Maddock, J., Aylward, M., & Oswald, I. (1977). Relationship between nocturnal plasma oestrogen concentration and free plasma tryptophan in perimenopausal women. *J Endocrinol, 72*(3), 395-396.

Tuomisto, J., & Tukiainen, E. (1976). Decreased uptake of 5-hydroxytryptamine in blood platelets from depressed patients. *Nature, 262*(5569), 596-598.

van der Waals, F. W., Mohrs, J., & Foets, M. (1993). Sex differences among recipients of benzodiazepines in Dutch general practice. *Bmj, 307*(6900), 363-366.

Van Goozen, S. H., Wiegant, V. M., Endert, E., Helmond, F. A., & Van de Poll, N. E. (1997). Psychoendocrinological assessment of the menstrual cycle: the relationship between hormones, sexuality, and mood. *Arch Sex Behav, 26*(4), 359-382.

van Praag, H. M. (1977). The Harold E. Himwich Memorial Lecture. Significance of biochemical parameters in the diagnosis, treatment, and prevention of depressive disorders. *Biol Psychiatry, 12*(1), 101-131.

van Praag, H. M. (1981). Management of depressionwith serotonin precursors. *Biological Psychiatry, 16*(291-310).

van Praag, H. M., & de Haan, S. (1979). Central serotonin metabolism and frequency of depression. *Psychiatry Res, 1*(3), 219-224.

Watson, N. R., Studd, J. W., Savvas, M., & Baber, R. J. (1990). The long-term effects of estradiol implant therapy for the treatment of premenstrual syndrome. *Gynecol Endocrinol, 4*(2), 99-107.

Weiss, S. R., & Post, R. M. (1998). Kindling: separate vs. shared mechanisms in affective disorders and epilepsy. *Neuropsychobiology, 38*(3), 167-180.

Weissman, M. M., Gammon, G. D., John, K., Merikangas, K. R., Warner, V., Prusoff, B. A., & Sholomskas, D. (1987). Children of depressed parents. Increased psychopathology and early onset of major depression. *Arch Gen Psychiatry, 44*(10), 847-853.

Weissman, M. M., Leaf, P. J., Holzer, C. E. d., Myers, J. K., & Tischler, G. L. (1984). The epidemiology of depression. An update on sex differences in rates. *J Affect Disord, 7*(3-4), 179-188.

Wickberg, B., & Hwang, C. P. (1997). Screening for postnatal depression in a population-based Swedish sample. *Acta Psychiatr Scand, 95*(1), 62-66.

Williams, W. A., Shoaf, S. E., Hommer, D., Rawlings, R., & Linnoila, M. (1999). Effects of acute tryptophan depletion on plasma and cerebrospinal fluid tryptophan and 5-hydroxyindoleacetic acid in normal volunteers. *J Neurochem, 72*(4), 1641-1647.

Wolfe, B. E., Metzger, E. D., & Jimerson, D. C. (1995). Comparison of the effects of amino acid mixture and placebo on plasma tryptophan to large neutral amino acid ratio. *Life Sci, 56*(17), 1395-1400.

Wurtman, J. J., Brzezinski, A., Wurtman, R. J., & Laferrere, B. (1989). Effect of nutrient intake on premenstrual depression. *Am J Obstet Gynecol, 161*(5), 1228-1234.

Wurtman, R. J. (1982). Nutrients that modify brain function. *Sci Am, 246*(4), 50-59.

Wurtman, R. J. (1987). Dietary treatments that affect brain neurotransmitters. In J. J. Wurtman (Ed.), *Annals of the New York Academmy of Sciences, Vol. 499 - Human Obesity* (pp. 179-190). New York: New York Academy of Sciences.

Wurtman, R. J., Hefti, F., & Melamed, E. (1981). Precursor control of neurotransmitter synthesis. *Pharmacological Reviews, 32*(4), 315-335.

Yatham, L. M., Barry, S., & Dinan, T. G. (1989). Serotonin receptors, buspirone, and premenstrual syndrome. *Lancet, 1*(8652), 1447-1448.

Young, S. N. (1991). Use of tryptophan in combination with other antidepressant treatments: a review. *J Psychiatry Neurosci, 16*(5), 241-246.

Young, S. N. (1993). The use of diet and dietary components in the study of factors controlling affect in humans: a review. *J Psychiatry Neurosci, 18*(5), 235-244.

Young, S. N., Ervin, F. R., Pihl, R. O., & Finn, P. (1989). Biochemical aspects of tryptophan depletion in primates. *Psychopharmacology, 98*(4), 508-511.

Young, S. N., & Gauthier, S. (1981). Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry, 44*(4), 323-327.

Young, S. N., Smith, S. E., Pihl, R. O., & Ervin, F. R. (1985). Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology, 87*(2), 173-177.

Zweifel, J. E., & O'Brien, W. H. (1997). A meta-analysis of the effect of hormone replacement therapy upon depressed mood. *Psychoneuroendocrinology, 22*(3), 189-212.

Appendix 1:

SUBJECT RECRUITMENT ADVERTISMENT

McGill University &
The Royal Victoria Hospital
seek

Menopausal women

(aged 40-60)
not currently receiving hormone therapy
to participate in a study examining:

The influence of hormones and dietary factors on mood in women.

This research project is directed by
Dr. B. Sherwin and L.A. Schleifer, M.A.
with Dr. S. Young & Dr. C. Benkelfat
Participants will receive $120 to
compensate for lost work time and
transportation costs.
For more information, please contact:
Laura Schleifer at 398-8595

Appendix 2:

Information and Consent Form

The Role of Estrogen in the Mood Altering Effects of Acute Tryptophan Depletion in Menopausal Women

INFORMATION AND CONSENT FORM

A. Information:

General: We are conducting a research study in order to investigate hormonal and dietary influences on mood in women. Previous research has shown that estrogen enhances mood, and that decreases in estrogen at different phases of the life cycle (e.g., premenstrually) lowers mood, but we do not fully understand how this occurs. Use of the tryptophan depletion technique (which temporarily lowers mood) may lead to a clearer understanding of these mechanisms. This type of information might eventually allow us to prevent depressive episodes in women and/or might lead to better treatment for women with depressive illness.

Estrogen Replacement Therapy (ERT): Between the ages of 48-52 years, the ovaries stop producing estrogen almost entirely. The most common short-term consequences of the decrease in estrogen production are symptoms such as hot flushes and night sweats, irritability, fatigue, and vaginal dryness. In most cases, ERT effectively reverses these symptoms. It may also be important to consider that long-term estrogen therapy protects against the development of osteoporosis (thinning of the bones that can result in fractures) and against coronary heart disease in older women.

The decision to begin taking ERT is an individual one and factors that should be considered include the particular woman's symptoms, medical history, family history, and lifestyle. The major contraindication to ERT is the presence of breast cancer.

While there are now many different estrogen preparations available on the market, Premarin (Wyeth-Ayerst Laboratories, Canada), the estrogen tablets we will use in this study, is the oldest and the most frequently prescribed estrogen drug worldwide.

Tryptophan, Estrogen and Mood: Tryptophan, an essential amino acid, is one of the building blocks of protein. It is present in the foods that make up a normal diet. Like estrogen, it is also thought to be involved in the regulation of mood. We intend to temporarily lower tryptophan levels using a tryptophan-free amino acid drink. This technique sometimes results in a lowering of mood, but not always. The purpose of this

study is to examine whether or not this lowering of mood occurs in women receiving HRT.

Procedure: A group of 72 healthy peri- and postmenopausal women who are not taking any psychotropic medication at present will be chosen for this study. Each participant will be seen by a gynecologist to establish that she has no contraindications to estrogen replacement therapy. If the participant has been examined by her own gynecologist within the previous six months, we will obtain written confirmation from him/her that no contraindications to estrogen therapy exist.

A preliminary interview will be conducted by a doctoral candidate in clinical psychology. This will involve a general psychiatric interview, an interview assessing family history of psychiatric disorders and other health problems, and the completion of several questionnaires assessing reproductive history and current mood state. A sample of blood will also be taken (approximately 4 teaspoons). In total, this will take approximately 3-4 hours. Then, participants will receive daily estrogen tablets, or inactive tablets, for 3 months. There is a 50% chance of being assigned to either group. Neither the investigator, nor the participant, will know which treatment group they have been assigned to until completion of the study. The hormone pills will be provided free of charge.

Towards the end of the treatment period, participants will be asked to come to the Research and Training Building of the Department of Psychiatry on two separate test days, one week apart. On the day before each test day, participants will be provided with a low protein diet which will either be delivered to their home, or picked up. On one test day, participants will be given a drink containing all of the amino acids normally found in protein; on the other test day, the drink will contain all of the amino acids except tryptophan. Neither the investigator nor the participant will know which drink they are ingesting on a given test day. These drinks occasionally cause nausea or "bloated" feelings for a short time, but in most cases there are no side effects. On both test days, each participant will be asked to complete questionnaires designed to evaluate mood, to perform a short computer task, and to give two samples of blood (approximately 4 teaspoons each). At the end of each test day, participants will be given a 1 gm supplement of tryptophan with a balanced meal. During test days, a psychiatrist will be on-call at all times. In total, testing will take approximately 7-8 hours per day.

Participation in this study is completely voluntary and all participants will be free to withdraw from the study at any time.

Confidentiality: All of the information gathered during this study will remain completely confidential. No names will appear on test materials, only participant identification numbers.

Study Investigators: If participants have questions at any time, they will be encouraged to contact the investigators, and they can be reached at the following phone numbers:

Principal Investigators		**Collaborators**	
Dr. Barbara B. Sherwin	398-6087	Dr. Simon Young	398-7317
Laura A. Schleifer, M.A.	398-8595	Dr. Chawki Benkelfat	398-6732
Department of Psychology McGill University		Department of Psychiatry McGill University	

B. Consent

I, _____, consent to participate in a study carried out under the auspices of the McGill University Departments of Psychology and Psychiatry. I have had the project explained to me by _____ and it has been explained that:

I. The purpose of the study is to improve our knowledge of the roles of estrogen, tryptophan, and their interaction in relation to mood in women. More specifically, we will be examining the possiblity that estrogen might prevent the lowering of mood that is sometimes associated with tryptophan depletion.

II. I will be asked to complete an interview which will evaluate my general health, my psychiatric and reproductive history, and the psychiatric and medical histories of my family. I will also undergo a routine gynecological examination. It has been explained that all information, including any information obtained during the study, will remain confidential and that my name will be removed from test material after completion of the study. It has also been explained that only group results will be reported.

III. If I decide to participate in the study, I will receive either an estrogen tablet or an inactive tablet daily for three months. The dose of estrogen used will be one most commonly prescribed for the treatment of postmenopausal women. Some women experience mild breast tenderness on this dose, but most have no side effects whatsoever. It has been explained that in menopausal woman with an intact uterus, a progestin is often added to the regimen in order to protect against endometrial hyperplasia, but that the administration of estrogen alone for 3 months does not represent a risk for hyperplasia. Following completion of the study, if I have been receiving estrogen and if I elect to continue with HRT, a progestin will be added to my regimen. If I have been receiving the inactive tablet, I will be offered active treatment. During the course of the study, I will receive the hormone pills free of charge.

IV. I will be asked to eat meals provided by the researchers the day before both test days, and I will be asked to come to the research facility at 9:00 a.m. the morning of the study, without having eaten since midnight. The study will finish at approximately 4:30 p.m.

V. In the laboratory, I will be asked to complete some pencil and paper tests, I will be weighed and measured and a sample of my blood will be taken (approximately 4 teaspoons), then I will be given a *milk-shake-type* drink containing various amino acids for breakfast. Amino acids are the building blocks of protein; the amount of amino acids contained in the shake will be about the same as that contained in a large steak. Five hours later, I will be given some additional pencil and paper tests, I will complete a short computer task, and a second blood sample will be taken. I will then be given a balanced meal and a tryptophan supplement before I leave.

VI. It has been explained that I may experience mild feelings of depression at some time during one or both test days, and that there is a slight risk that these feelings may persist beyond the immediate duration of the actual test day. In the unlikely event that this happens, I will receive the appropriate care.

VII. It has been explained that I will be compensated for lost work time and transportation costs in the following manner: (1) initial interview: $30, (2) study day 1: $40, (3) study day 2: $50. If I decide to withdraw from the study during the initial interview, or should the investigators decide that I do not satisfy the inclusion criteria, I will be compensated $30. If I decide to withdraw during one of the study days, I will get compensated for that day but no further compensation.

VIII. It has been explained that the only advantage to me of participating in this study is that I will be provided with an opportunity to learn about hormone replacement therapy and to make an informed decision regarding future treatment. This information will be provided by Laura Schleifer during the initial screening and at any other time questions arise. It has also been explained that information from this type of study may eventually be useful in preventing or inhibiting the onset of mood disorders and/or reproduction related mood changes, in designing better treatments for these disorders and in designing better treatment for menopausal women in general.

IX. The inconvenience to me is the time spent during the clinical evaluation (about 4 hours) and the study sessions (approximately 7 hours per day for 2 days) and the discomfort of having blood drawn. Additionally, I am aware that the ingestion of the amino acid drink may sometimes result in one or more of the following effects: (1) feelings of nausea or "feeling bloated", which usually subside in one to three hours, (2) lowered or heightened energy levels (3) drowsiness or tiredness, or heightened alertness, and (4) a possible change in mood. These effects are generally short-lasting and are usually completely reversed following the balanced meal and tryptophan supplement given at the end of each study day. It is possible (although very unlikely) that I may experience some of the above symptoms for a longer duration than the study day. In this case, I will remain at the laboratory for a short time under medical observation. I will then be driven home by taxi if required, at the study's expense. I will receive a follow-up telephone call that evening and on subsequent days to see if the symptoms(s) is (are) still present, and if so, I will be asked to meet with the investigators for further evaluation.

X. It has been explained that participation in this study is voluntary, that I am assured of receiving medical care, including hormone treatment, whether or not I participate, and that I may discontinue participation at any time.

XI. If I have any questions about this research, it has been explained that my questions will be answered by either Laura Schleifer or her associates and they may be reached at the above-listed numbers. If I have any questions regarding patient's rights, it has been explained that my questions will be answered by the patient representative, who can be reached at 842-1231 (ext. 5655).

XII. I acknowledge that I have received a complete copy of this information and consent form.

Signature	**Date**
Witness	**Date**
Investigator	**Date**

Appendix 3:

Reproductive and Medical History Questionnaire

Reproductive & Medical History Questionnaire

A. Menstruation

1. Age at last menstrual period (years/months)_____
2. Age of onset of first menstrual period (years/months): _____ _____
3. Age when menstrual periods became *ir*regular (years/months): _____

4. Have you had past menstrual irregularities? **Yes / No** (if yes, please specify)

_____

_____

_____

5. If you have experienced ***fluctuations*** in any of the following symptoms ***over the course of the menstrual cycle prior to the peri-menopausal period***, please rate their severity using the following scale and their frequency by indicating how many menstrual cycles per year:

Severity:
1= barely noticeable
2= mild
3= moderate
4= severe
5= incapacitating

a) depressed mood, feelings of hopelessness, or self-critical thoughts:
severity?____ frequency?____/year

b) anxiety, tension, feelings of being "keyed up", or "on edge":
severity?____ frequency?____/year

c) rapidly changing mood (e.g. feeling suddenly sad or tearful or increased sensitivity to rejection):
severity?____ frequency?____/year

d) persistent and marked anger or irritability or increased interpersonal conflicts:
severity?____ frequency?____/year

e) decreased interest in usual activities (e.g. work, school, friends, hobbies):
severity?____ frequency?____/year

f) difficulty concentrating:
severity?____ frequency?____/year

g) lethargy, easily tired, or marked lack of energy:
severity?____ frequency?____/year

h) marked change in appetite, overeating, or specific food cravings:
severity?____ frequency?____/year

(if specific food cravings, which?_____)

i) excessive sleeping or insomnia:
severity?____ frequency?____/year

j) a sense of being overwhelmed or out of control:
severity?____ frequency?____/year

k) other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, a sensation of "bloating", and/or weight gain:
severity?____ frequency?____/year

6. If you have experienced fluctuations in any of the above symptoms over the menstrual cycle:

a) when did they typically **occur** ? (if you choose more than one, please specify)

During menstruation:_____
During the week following menstruation: _____
During the week preceding menstruation: _____
Throughout the menstrual cycle: _____

b) when did they typically **stop**? (if you choose more than one, please specify)

During menstruation:_____
During the week following menstruation: _____
During the week preceding menstruation: _____

7. Did the occurrence of any of these symptoms interfere with work, school, usual social activities and/or relationships with others (e.g. avoidance of social activities, decreased productivity and efficiency at work or school)? **Yes / No** (if yes, please specify)

_____
_____
_____

8. Have you ever sought treatment, or taken any medication for ***pre***menstrual problems?
Yes / No (if yes, please specify)

_____
_____
_____

9. If you have ever experienced dysmenorrhea (cramps or pain) during menstruation, please rate its frequency and severity using the above scale:
severity?____ frequency?____/year

10. Have you ever sought treatment or taken any medication for menstrual problems (e.g.cramps, excessively heavy flow, clots)? **Yes / No** (if yes, please specify)

_____
_____
_____

B. Oral Contraception & Hormone Use

11. Have you ever used oral contraceptives and/or hormones for any reason? **Yes / No** (if no, why not? if yes, please specify type, duration, and ages used)

_____
_____
_____

12. If you did use oral contraceptives and/or hormones, why did you stop?

_____
_____
_____

13. Did you experience ***any*** side effects while taking hormones (e.g. headaches, acne, depression, irritability etc.)? **Yes / No** (if yes, please specify)

_____
_____
_____

C. Pregnancy and Postpartum

14. Number of pregnancies carried full term: _____

15. Total number of months nursing:_____

16. Number of pregnancies not carried full term: _____

17. Did you ever experience feelings of sadness, tearfulness or irritability during the week following delivery? **Yes / No** (if yes, please describe, including frequency)

_____
_____
_____

18. Did you ever experience a more long term mood disturbance beginning 2-27 weeks following delivery? **Yes / No** (if yes, please describe, including frequency)

_____

_____

_____

19. Did you ever seek treatment or medication for a mood disturbance following delivery? **Yes / No** (if yes, please specify)

_____

_____

_____

D. General Medical History

20. Do you have or have you ever sought treatment for any of the following medical conditions or illnesses (please specify where appropriate):

a)	diabetes	**Yes / No**	_____
b)	thyroid disorder	**Yes / No**	_____
c)	heart condition	**Yes / No**	_____
d)	high/low blood pressure	**Yes / No**	_____
e)	high cholesterol	**Yes / No**	_____
f)	cancer	**Yes / No**	_____
g)	other	**Yes / No**	_____

21. Do you have or have you ever sought treatment for any of the following psychological conditions or illnesses (please specify where appropriate):

a)	anxiety (including phobias)	**Yes / No**	_____
b)	depression	**Yes / No**	_____
c)	hallucinations/schizophrenia	**Yes / No**	_____
d)	alcoholism	**Yes / No**	_____
e)	drug abuse	**Yes / No**	_____
f)	other	**Yes / No**	_____

22. Do any of your family members have, or have they ever sought treatment for, any of the above medical or psychological conditions or illnesses? **Yes / No**
(if yes, please specify, including relationship [e.g. mother, father, son, aunt, etc.])

_____

_____

_____

_____

_____

_____

Appendix 4:

Menopausal Index

Below are a list of symptoms sometimes reported by menopausal women. Please indicate the degree to which you are now experiencing each of these symptoms by circling the most appropriate number on the scale. Remember, we are interested in how you are experiencing these symptoms NOW.

1. **Hot Flashes:** almost never 0 1 2 3 4 5 6 7 very often

2. **Cold Sweats:** almost never 0 1 2 3 4 5 6 7 very often

3. **Feelings of Suffocation:** almost never 0 1 2 3 4 5 6 7 very often

4. **Weight Gain:** almost never 0 1 2 3 4 5 6 7 very often

5. **Rheumatic Pains:** almost never 0 1 2 3 4 5 6 7 very often

6. **Cold Hands & Feet:** almost never 0 1 2 3 4 5 6 7 very often

7. **Numbness & Tingling:** almost never 0 1 2 3 4 5 6 7 very often

8. **Breast Pain:** almost never 0 1 2 3 4 5 6 7 very often

9. **Unusually Heavy Menstrual Flow:** almost never 0 1 2 3 4 5 6 7 very often

10. **Skin Crawls:** almost never 0 1 2 3 4 5 6 7 very often

11. **Tired Feelings:** almost never 0 1 2 3 4 5 6 7 very often

12. **Headaches:** almost never 0 1 2 3 4 5 6 7 very often

13. **Pounding of the Heart:** almost never 0 1 2 3 4 5 6 7 very often

14. **Dizzy Spells:** almost never very often
0 1 2 3 4 5 6 7

15. **Irritability & Nervousness:**
almost never very often
0 1 2 3 4 5 6 7

16. **Blue & Depressed Feelings:**
almost never very often
0 1 2 3 4 5 6 7

17. **Forgetfulness:** almost never very often
0 1 2 3 4 5 6 7

18. **Trouble Sleeping:** almost never very often
0 1 2 3 4 5 6 7

19. **Difficulty Concentrating:** almost never very often
0 1 2 3 4 5 6 7

20. **Crying Spells:** almost never very often
0 1 2 3 4 5 6 7

21. **Blind Spots Before the Eyes:**
almost never very often
0 1 2 3 4 5 6 7

22. **Excitability:** almost never very often
0 1 2 3 4 5 6 7

23. **Attacks of Panic:** almost never very often
0 1 2 3 4 5 6 7

24. **Loss of Interest in:** almost never very often
Most Things 0 1 2 3 4 5 6 7

25. **Tense or Wound Up Feelings:**
almost never very often
0 1 2 3 4 5 6 7

26. **Worrying Needlessly:** almost never very often
0 1 2 3 4 5 6 7

27. **Pressure or Tightness:** almost never very often
in the head or body 0 1 2 3 4 5 6 7

Appendix 5:

Low-Protein Diet

Pre-Test Low-Protein Diet

Breakfast:

- Two bananas
- Two slices of white bread
- One glass of orange juice

Lunch:

- Iceberg lettuce
- One tomato
- One cucumber
- One stock of celery
- One medium Carrot
- One apple
- One pear
- One twix chocolate bar
- One small box of raisins

Dinner:

- Stir-fried Vegetables (pre-packaged frozen dinner)

Snacks:

- One apple sauce
- One pear
- One small box of raisins
- One twix chocolate bar

Low Protein Diet

		Weight (g)	Protein (g)	Fat (g)	Carb. (g)	Kcal
Breakfast:	2 bananas	228	2.4	2	54	210
	½ cup orange juice	120	0.8	0	13	52
	2 slices white toast	42	0	2	24	128
	margarine	10	0	8	0	68
	jelly	42	0	0	30	116
	decaf coffee or tea		0.5	0	0	
	½ & ½ cream	20	0	2	1	27
	2 packages sugar	8		0	8	32
Lunch:	shredded lettuce	80	0.7	0	2	10
	raw carrots	55	0.6	0	5	23
	1 stalk raw celery	40	0.3	0	2	6
	1 tomato	123	1.3	0	6	27
	½ cup cucumber	52	0.3	0	2	7
	1 tbsp oil	15	0	14	0	129
	1 package vinegar	20	0	0	0	1
	1 package raisins	45	1.5	0	36	136
	1 apple	140	0	0	21	82
	1 peach	90	0.6	0	10	38
	1 twix chocolate bar	48	1.0	6	16	118
	Decaf coffee or tea		0	0	0	
	½ & ½ cream	20	0.5	2	1	27
	2 packages sugar	8	0	0	8	32

Dinner:	Stir Fried vegetables:					
	4 tbsp onions	40	0	0	3	12
	carrots	55	0.5	0	4	17
	1 stalk celery	40	0.3	0	1	6
	½ cup broccoli	44	1.4	0	2	11
	½ cup cauliflower	50	1.2	0	2	11
	½ cup mushrooms	35	0.9	0	1	39
	½ cup green pepper	50	0	0	3	13
	3 tbsp oil	45	0	42	0	386
	½ cup applesauce	128	0.2	0	25	97
	decaf coffee or tea		0	0	1	
	½ & ½ cream	20	0.5	2	8	27
	2 packages sugar	8	0	0	10	32
	1 peach	90	0.6	0		38

Snacks:	1 package raisins	45	1.5	0	36	136
	1 twix chocolate bar	48	1.0	6	16	118

Total:	23.6	36	351	2212

Appendix 6:

Physician information letter

April, ____

Dear Dr. _____

We are conducting a study to examine the effects of estrogen on mood in menopausal and peri-menopausal women. This study is a joint project of the McGill University Departments of Psychology and Psychiatry and it has received ethical approval from the Institutional Review Board, Faculty of Medicine, McGill University. Funding is provided by the Medical Research Council of Canada through a grant awarded to Dr. B. Sherwin.

Your patient, Ms. _____ meets the selection criteria for this study. If you have examined her within the last year and if she had no contraindications to estrogen replacement therapy (please see attached list of exclusion criteria), we would appreciate it if you would sign the enclosed form and attach a **3 month prescription for Premarin 0.625 mg or placebo** in the patient's name. The Royal Victoria Hospital pharmacist will randomly assign participants to treatments. Please return both to LA. Schleifer, either in an addressed-stamped envelope that has been provided or via the patient.

We would greatly appreciate your timely response in this matter as any delay will interfere with the research protocol. If you have any questions, please feel free to contact us.

We thank you in advance for your cooperation.

Your truly,

Barbara B. Sherwin, Ph.D.
Professor
Departments of Psychology
and OB/GYN
McGill University
and Jewish General Hospital

398-6087

Laura A. Schleifer, M.A.
Doctoral Candidate in Clinical
Psychology
McGill University

398-8595

Contraindications to Estrogen Replacement Therapy:

(1) unexplained vaginal bleeding
(2) active liver disease
(3) carcinoma of the breast
(4) active vascular thrombosis
(5) a first degree relative with a history of breast cancer

Appendix 7:

PHYSICIAN CONSENT FOR PARTICIPATION

July 1, _____

L.A. Schleifer, M.A. and B.B. Sherwin, Ph.D.
Department of Psychology
McGill University
1205 Dr. Penfield Avenue
Montreal, Quebec
H3A 1B1

Dear Ms. Schleifer and Dr. Sherwin:

I last examined my patient_____
(please print full name of patient)
on _____. She has no contraindications to
(please print date)
estrogen replacement therapy and could receive either estrogen or placebo in your study.

Please find enclosed a three month prescription in her name for Premarin 0.625 mg or placebo.

Yours truly,

_____ _____
Name **Signature**

_____
Date