Early Development of Dietary Supplements for Preventing Postpartum Depression

by

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Abstract

Background: Postpartum depression (PPD) is the most common complication of childbearing with 13% prevalence. A dietary prevention strategy countering elevated monoamine oxidase-A (MAO-A) in early postpartum might be effective, since MAO-A is elevated by 43% early in postpartum, during healthy ranges of postpartum blues (PPB). Since greater severity of PPB increases risk for PPD, the aim of this thesis was to develop a widespread intervention based on tryptophan, tyrosine and blueberry extract. First we proposed to assess the effect of different components upon breast milk. Subsequently we aimed to develop a method to optimally quantify severity of PPB using the depressed mood induction procedure (MIP). At last, the ability of optimal doses of tryptophan, tyrosine and blueberry extract was investigated in reducing the intensity of PPB.

Methods: 24 healthy breastfeeding women were randomly assigned to 2g, 5g, 10g tyrosine or no supplement, and 30 were randomly assigned to 2g, 4g tryptophan, 20g, 40g alpha-lactalbumin or no supplement. Four breast milk and seven blood samples were collected.
For PPB quantification, 45 healthy women were recruited (23 vulnerable to sad mood and 22 non-vulnerable) and mood measures were completed before and after MIP. Finally, 26 healthy day-5 women were recruited into 2 groups: receiving dietary supplement (10g tyrosine, 2g tryptophan and blueberry extract/juice) or no supplement. Severity of PPB was measured before and after MIP.

**Results:** Oral tyrosine, tryptophan and alpha-lactalbumin did not increase total tyrosine or tryptophan contents of breast milk ($p=0.074$ (trend to decrease), $p=0.232$ respectively) and plasma levels rose significantly ($p<0.005$). Visual analog scale mood scores were strongly raised after MIP in women vulnerable to sad mood ($p<0.005$). At last, the dietary supplement combination was significantly successful in attenuating the effect of sad MIP in those receiving the supplement ($p<0.001$).

**Conclusion:** The negligible effect of oral tyrosine and tryptophan on its concentration in breast milk supported their further development as part of a prevention strategy for PPD. At the level of open trial, the results support the ability of a dietary supplement intervention based on 2g tryptophan, 10g tyrosine and blueberry extract/juice to virtually eliminate the intensity of PPB.
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Contributions

Yekta Dowlati (author) solely prepared this thesis. All different aspects of each study presented, including development of overall research plan, study design, preparation, regulatory applications, project conception, recruitment, interviews, implementation, data analysis, and writing of all original research and publications was performed in whole or in part by the author.

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Dr. Jeffrey H. Meyer (Primary Supervisor) - Mentorship, providing resources, development of overall research plan, guidance and assistance in planning, project conception, data analyses, manuscript and thesis preparation.

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Dr. Donna E. Stewart - Scientific consultation, final contents of manuscripts in Chapters 2, 3 & 4.

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List of Abbreviations

5-HT   5-Hydroxytryptamine (Serotonin)
5-HT1A  Serotonin 1A Receptor
α-LAC  Alpha-lactalbumin
ACTH  Adrenocorticotropic Hormone
AMPT  Alpha Methyl-Para-Tyrosine
ANCOVA  Analysis of Covariance
ANOVA  Analysis of Variance
APTD  Phenylalanine Tyrosine Depletion
ATD  Acute Tryptophan Depletion
BDI  Beck Depression Inventory
BDNF  Brain Derived Neurotrophic Factor
CBT  Cognitive Behavioral Therapy
CRH  Corticotropin Releasing Hormone
DAS  Dysfunctional Attitude
DHA  Docosahexaenoic Acid
DSM  Diagnostic and Statistical Manual
EPA  Eicosapentaenoic Acid
EPDS  Edinburgh Postnatal Depression Scale
GABA  Gama Aminobutyric Acid
H2O2  Hydrogen Peroxide
HDRS  Hamilton Depression Rating Scale
HPA  Hypothalamic Pituitary Adrenal
HSP  Hormone Simulated Pregnancy
IPT  Interpersonal Therapy
LNAA  Large Neutral Amino Acids
LSD  Fisher's least significant difference
MAO-A  Monoamine Oxidase-A
MAO-A VT  Monoamine Oxidase-A Total Distribution Volume
MAO DV  Monoamine Oxidase-A Distribution Volume
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
</tr>
<tr>
<td>MDE</td>
<td>Major Depressive Episode</td>
</tr>
<tr>
<td>MIP</td>
<td>Mood Induction Procedure</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>POMS</td>
<td>Profile of Mood State</td>
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<tr>
<td>PPD</td>
<td>Postpartum Depression</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid Eye Movement</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM Disorders</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin Transporter</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>SWS</td>
<td>Short Wave Sleep</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
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Chapter 1    Introduction
1.1. Importance of Postpartum Depression and the Need for a New Method of Prevention

1.1.1. Importance of Postpartum Depression (Major Depressive Episode with Postpartum onset)

Major Depressive Disorder (MDD) is the leading cause of death and disability for women aged 15-44 worldwide (World Health Organization 2008). Postpartum depression (PPD), defined as a clinical level major depressive episode (MDE) starting within the first year after giving birth, is highly impactful, being the most common complication of childbearing with a prevalence rate of 13% (Kornstein 2001; NICE clinical guideline 2007; O'Hara 2009; O'Hara and Swain 1996; Pop et al. 1993; Steiner 1998). Postpartum depression can lead to serious consequences: During the first postpartum year, presence of psychiatric illness is associated more than a 10 fold risk of suicide (Comtois et al. 2008) and women who have experienced PPD are at greater risk of having further MDE both following future deliveries and at times unrelated to childbirth (Goodman 2004; Kumar and Robson 1984; Nott 1987; Philipps and O'Hara 1991; Warner et al. 1996; Wisner et al. 2002). Major depressive episode with postpartum onset may last for years (Goodman 2004; Horowitz and Goodman 2004). Also, PPD may be associated with cognitive delay, emotional and behavioral difficulties in children of mothers suffering from PPD and can also have negative impact on the health of other children in the family and the partner (NICE clinical guideline 2007; O'Hara 1994).
Risk for PPD is influenced by clinical history, as well as social and psychological factors. The three elements of clinical history which are particularly strong factors are a previous history of MDEs (in postpartum or at other times), a history of symptoms at a sub-clinical level in the third trimester and severity of postpartum blues (Adewuya 2006; Astbury et al. 1994; Hannah et al. 1992; O'Hara et al. 1991b). In general, meta-analyses have identified few moderate to strong risk factors for PPD, including previous history of MDEs, depression and anxiety during pregnancy, postpartum blues, stressful life events, low social support and neuroticism (Beck 2001; O'Hara and McCabe 2013; O'Hara and Swain 1996; Robertson et al. 2004). Risk factors with smaller association may include: low socioeconomic status and marital status. A few studies have also investigated obstetrical complications and neonatal outcomes as risk factors for PPD, with small to moderate effects reported in some studies (Beck 2001; Nakku et al. 2006; Norhayati et al. 2015; O'Hara and McCabe 2013; Robertson et al. 2004); however the association is somehow currently inconclusive. Most positive results for the association of obstetrical complications and PPD have been reported in developing countries. Investigational studies on neonatal outcome as a risk factor for PPD have shown positive findings in some, but not all studies. Some studies have found that women whose infants had medical illnesses, are born prematurely and are temperamentally difficult are likely to experience PPD (Beck 2001; O'Hara and McCabe 2013; Robertson et al. 2004; Vigod et al. 2010). Gender of baby has been identified as a risk factor in some developing countries as well (Hassanein et al. 2014).

One potentially important predictive variable is maternal sleep disturbances in early postpartum, that has received relatively less attention. Sleep in the postpartum period is fragmented and inefficient, characterized by several and frequent awakenings, as women
adapt to new demands of motherhood. For some mothers is harder to adapt to these changes. Sleep disturbances may result in negative consequences, such as dysphoric mood, fatigue, irritability and impaired cognitive function (Dorheim et al. 2009). Subjective sleep assessments reporting poor sleep quality have been reported to be associated with higher risk of developing PPD (Dennis and Ross 2005; Park et al. 2013). It should be noted that there is a bidirectional causal relationship between the phenomena of sleep disturbance and PPD (Dorheim et al. 2009; Swanson et al. 2011).

1.1.2. Mood Disturbances in the Postpartum Period

There are three types of mood disturbances in the postnatal period:

One is the “baby blues” or postpartum blues which is common (occurring up to 75% of the time) and transient occurring within the first week postpartum (O'Hara 1994; O'Hara et al. 1991a). Since there are no agreed criteria for postpartum blue, prevalence rates range from anywhere between 25% and 85% (O'Hara et al. 1991b). Symptoms typically begin around 3 days after delivery and usually resolve within 10 days; however, some individuals continue to PPD (Kendell et al. 1981). Postpartum blues is a syndrome of sadness that is usually accompanied by fatigue, anxiety, mood lability, crying, irritability, sleep disturbances, and poor appetite. Emotional lability is the hallmark symptom of postpartum blues (O'Hara et al. 1991b). Although extremely common, there are no well-established diagnostic criteria or rating scale that addresses all negative aspects of postpartum blues and most scales used are nonspecific. The etiology of postpartum blues is not well understood, but it is more believed
that withdrawal from estradiol and progesterone during childbirth are one of the main biological causes (Bloch et al. 2003).

The second is MDE with postpartum onset or “postpartum depression”, which is defined as a MDE that occurs within the first four weeks after delivery. The Diagnostic and Statistical Manual of Mental Disorders-Fourth edition (DSM-IV) assigns a “with postpartum onset” specifier to episodes of depression that begin within four weeks of delivery. Though, a time frame of 1 year has been defined for PPD in many research studies and in clinical practice. According to the DSM-IV (APA 2000), at least one core symptom (depressed mood or anhedonia) along with five of nine depressive symptoms from criteria (such as fatigue, feelings of worthlessness or guilt, lack of ability to concentrate, suicidal ideation, or significant changes in weight or sleep) must be present for at least 2 weeks to diagnose a MDE (APA 2000). While there are many risk factors for PPD, the severity of postpartum blues is a consistent and robust predictor (Adewuya 2006; Hannah et al. 1992; O'Hara et al. 1991a). Presence of reasonably severe postpartum blues raises the risk of PPD about four fold (Adewuya 2006; O'Hara et al. 1991a).

The third is postpartum psychosis (which may be accompanied by depressive symptoms). This condition is rare, occurring in 0.1 to 0.2% of deliveries and is strongly associated with presence of bipolar disorder (Brockington et al. 1981; Kendell et al. 1987).

In addition, two other mood disturbances that can be observed in postpartum are mania and hypomania. Childbirth is also a time of elevated risk for the onset or recurrence of hypomanic or manic episodes. The prevalence of manic and hypomanic episodes significantly increase in the postpartum compared to pregnancy period. Women with a history of bipolar disorder or pre-existing diagnosis are at particularly high risk of a mood
episode in the postpartum period. A Scandinavian case registry study has estimated a 23-fold increase in the risk of bipolar disorder admission in the postpartum period (Munk-Olsen et al. 2006). The consequences of misdiagnosis can be particularly serious as treatment with antidepressants may precipitate mania, a mixed state, or rapid cycling and thus raise the risk for psychiatric hospitalization.

Estimates of the prevalence of postpartum hypomania in non-clinical populations have ranged from 9.6% to 20.4% on day 3 postpartum, with symptoms being evident from day 1 postpartum (Glover et al. 1994; Heron et al. 2009; Lane et al. 1997; Sharma et al. 2009). Symptoms are similar to non-postpartum hypomania and include elation, increased goal-directed activity, over-talkativeness, racing thoughts, decreased sleep requirement, distractibility, and irritability. Since prevalence of hypomania in the first week of postpartum has been shown to be as much as 8 folds compared to pregnancy, it is thought that childbirth is a strong trigger (Heron et al. 2009). While hypomanic symptoms alone do not cause pronounced impairment in social or occupational functioning, it has been shown to be associated with increased risk of developing PPD (Lane, 1997). Moreover, the rather unique circumstances accompanying childbirth and normal joy experienced may make it difficult to correctly diagnose hypomania. Unless enquired particularly, most women may fail to report hypomanic symptoms and focus instead on symptoms of postpartum blues or PPD.

This thesis relates to the postpartum blues and postpartum depression.
1.1.3. Postpartum Depression Treatment

1.1.3.1. Need to Treat Postpartum Depression

Postpartum depression is a critically important medical problem and treatment is significantly essential. Untreated PPD often persists for a long time and more importantly is associated with a much greater risk of suicide (Comtois et al. 2008; Goodman 2004). Results from longitudinal studies have shown that in a considerable percentage of women, PPD may become chronic and continue to stay for months and years after delivery (Goodman 2004; Horowitz and Goodman 2004). On average, during pregnancy, the risk of suicide is lower and it elevates during postpartum, yet is still less than other times in life (Samandari et al. 2011). However, when psychiatric illness such as postpartum depression occurs, the risk of suicide elevates more than 10 fold (Comtois et al. 2008; Lewis 2001). It has negative impacts both on the mother and infant. It can incapacitate the mother and elevate the risk of future MDEs both following future deliveries and at time unrelated to childbirth (Goodman 2004). Evidence suggests its impact on spousal relationship, child care and subsequent health of infant and maternal suicide or infanticide (Grace et al. 2003; O'Hara 2009; Robinson and Stewart 2001).

Postpartum depression goes beyond influencing mother-infant bonding, mother’s positive interaction with her infant and response to infant’s basic needs. Maternal PPD is associated with short-term and long-term behavioral, cognitive and health related consequences for the child. As for behavioral consequences, untreated and persistent maternal depression is associated with greater likelihood of internalizing and externalizing disorders in offspring.
from childhood to adolescent, especially in those who have experienced PPD in the first 6 months after delivery (Hay et al. 2003; O'Hara and McCabe 2013; Weissman et al. 2006a). Many studies have pointed out that PPD is associated with 2 to 3 fold greater risk of MDD and anxiety disorders in the offspring (Weissman et al. 2006b). It has been shown that remission of maternal PPD after 3 months of antidepressant therapy has a positive effect on child’s anxiety and disruptive behavior and depressive disorders (Weissman et al. 2006a). As for cognitive outcomes, PPD predicts poorer language and IQ development, which can be evident from childhood to adulthood. For both behavioral and cognitive outcomes, rather than the presence of PPD, it appears that chronicity and severity of PPD is particularly important. As mentioned earlier, due to lack of maternal caretaking behaviour, her infant’s health may be affected as well. Poor weight gain, higher rates of colic, gastrointestinal and respiratory infections have been reported in infants of mothers suffering from PPD (Carter et al. 2001; Dennis and Ross 2005; Jacobsen 1999; Lee and Gotlib 1991; Sohr-Preston and Scaramella 2006; Weissman et al. 2006b). Furthermore, as offspring of depressed mother enters middle age, higher rates of medical problems, particularly cardiovascular problems, has been observed (Weissman et al. 2006b).

Given all these adverse sequelae of PPD on women’s health and function as well as the consequences on her infant, treatment is considerably important.
1.1.3.2. Role for Psychotherapy in Postpartum Depression

There is good evidence for the use of two non-pharmacological approaches in treating PPD, cognitive behavioral therapy (CBT) and interpersonal therapy (IPT) under certain circumstances (Dimidjian and Goodman 2009). Psychotherapy is usually effective short-term therapy for mild to moderate depression (Persons et al. 1996). A recent meta-analysis demonstrated that psychotherapy, including CBT and IPT, has a moderate effect on treating PPD with the mean standard effect of 0.61 (95% CI:0.37-0.85) (Cuijpers et al. 2008). Previous meta-analyses on the effectiveness of psychotherapy, psychosocial and psychological interventions, have also shown them to be an effective treatment for PPD (Bledsoe and Grote 2006; Dennis and Hodnett 2007). A study looking at the effect of IPT on PPD has found that 43.8% of women on IPT recovered from their depressive episodes compared to 13.7% controls based on Hamilton Depression Rating Scale scores (HAM-D) (O’Hara et al. 2000). Another study looking at the effect of CBT found that 66.6% of postpartum depressed mothers on CBT recovered compared to only 6.6% of controls based on HAMD scores (Chabrol et al. 2002). Long-term data of these therapies for preventing relapse/recurrence is limited. Generally for depression, successful CBT is associated with prevention of relapse/recurrence, lasting beyond the end of therapy (Paykel 2007).

While a clear advantage of CBT and IPT is the avoidance of medication, specifically when one is breastfeeding and therefore not concerned with infant exposure to antidepressants (Battle et al. 2008; Dennis and Chung-Lee 2006; Parker et al. 2006; Pearlstein et al. 2006), there may be circumstances in which antidepressant medication is preferred. While, most women with mild to moderate PPD may benefit from psychotherapy, there is a lack of
experience with this approach in women with high levels of suicidality. In general there is more experience with antidepressant treatment in moderate to severe PPD and MDE (American Psychiatric Association (APA) 2010; di Scalea and Wisner 2009). When there is some risk of suicide, and when PPD is moderate to severe, rapid response is very helpful given the additional demands of mothering an infant. Antidepressants tend to achieve maximal effect by 6 weeks; however, IPT and CBT take 12 and 18 weeks respectively, therefore length of time for response is a disadvantage for psychotherapy. Other reasons why psychotherapy might not be chosen include lack of availability of CBT and IPT, higher cost, and time commitment which may be difficult to combine with childcare requirements (Abreu and Stuart 2005).

1.1.3.3. Role for Pharmacological Treatment in Postpartum Depression

In clinical decision-making, treatment has priority (over antidepressant exposure from breastfeeding, for example) so as to avoid adverse sequelae of PPD like increased risk of suicide, long-term persistence of symptoms, and potential adverse effects upon infant development (Comtois et al. 2008; Goodman 2004; Macfarlane A. 2001). The main evidence for antidepressant treatment of PPD is the more general evidence of efficacy antidepressants in MDD, but for selective serotonin reuptake inhibitors (SSRI), efficacy is also demonstrated specifically in PPD (Appleby et al. 1997; Bloch et al. 2012; Hantsoo et al. 2014; Misri et al. 2004; Sharp et al. 2010; Wisner et al. 2006; Yonkers et al. 2008) (Table 1-1). While there is good evidence for CPT and IPT for MDE with mild to moderate severity of symptoms (Cuijpers et al. 2008; Persons et al. 1996), a more rapid response, which is frequently
obtainable with antidepressants, is often desirable when risk of the suicide is elevated. SSRIs have become the mainstay of treatment for moderate to severe postpartum depression, due to their less adverse effects and relative safety in overdose compared to tricyclic antidepressants (Wisner, 2002). The availability of antidepressants and their relatively rapid rate of response as compared to CBT and IPT have made them a common choice of treatment. The issue of preventing suicide through rapid treatment may be balanced against reports of associations of SSRIs with increased suicide attempts particularly in children, adolescents and, to some extent, young adults but the frequent response of MDE to SSRI outweighs this issue (Bridge et al. 2007). The current perspective of the Canadian Paediatric Society is that: “Postpartum use of SSRIs is not a contraindication to breastfeeding, and women who choose to breastfeed should be supported.”

Overall, there is substantial support for pharmacological treatment for PPD, hence, from the perspective of developing a dietary supplement to impact upon PPD, there is a lesser need to treat PPD. However, the need to prevent PPD with a dietary supplement is a different issue which will be detailed further in section 1.1.4.
### Table 1-1. Randomized Controlled Trials of Pharmacological Treatments in Postpartum Depression

<table>
<thead>
<tr>
<th>RCT</th>
<th>Duration</th>
<th>Number of PPD</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Wisner et al. (Wisner et al. 2006)  
• Sertraline (25-200 mg/d)  
• Nortriptyline (10-150 mg/d) | 8 weeks | 109 |  
• Proportion of responders or remitters and the time did not differ between the two groups.  
• Non-detectable or non-quantifiable infant serum levels.  
• No adverse effects in the infants.  
• Total side effect burden in mothers similar with both drugs. |
| Misri et al. (Misri et al. 2004)  
• Paroxetine (10-50 mg/d)  
• Paroxetine + 12 sessions CBT | 12 weeks | 35 |  
• Significantly effective improving in mood and anxiety symptoms and PPD treatment in both groups.  
• Combination of paroxetine with CBT was not superior to the AD alone and also the addition of CBT did not change the paroxetine dose needed for reaching remission. |
| Yonkers et al. (Yonkers et al. 2008)  
• Paroxetine (10-40 mg/d)  
• Placebo | 8 weeks | 70 |  
• Significant increase in remission rate in paroxetine group as compared to placebo after 8 weeks (37% vs. 14%).  
• Response rate higher in the paroxetine group but not significantly (43% vs. 32%).  
• Paroxetine dose was not different between responders and non-responders.  
• Rates of adverse effects were not statistically different among two groups. |
| Appleby et al. (Appleby et al. 1997)  
• Fluoxetine (20 mg/d) + 1 session counseling  
• Placebo + 1 session counseling  
• Fluoxetine (20 mg/d) + 6 sessions counseling  
• Placebo + 6 sessions counseling | 12 weeks | 87 |  
• Significant improvement in depressive symptoms in all groups, especially in the fluoxetine group.  
• No specific advantages for the combination of fluoxetine and counseling compared to any of the single treatments. |
<table>
<thead>
<tr>
<th>RCT</th>
<th>Duration</th>
<th>Number of PPD</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Sharp et al., (Sharp et al. 2010) |          |               | • Response rate significantly higher in antidepressant group compared to supportive care after 4 weeks (45% vs. 20%).  
• No difference at 18 weeks, due to the use of antidepressant by a portion of subjects in supportive care. |
| Hantsoo et al., (Hantsoo et al. 2014) |          |               | • Response rate higher in sertraline group compared to placebo (59% vs. 26%).  
• Remission rate higher in sertraline group compared to placebo (63% vs. 21%).  
• No adverse effects in the infants. |
| Bloch et al., (Bloch et al. 2012) |          |               | • Both groups improved significantly, but there were no significant difference between groups in response rate (70% vs. 55%).  
• Remission rate higher in sertraline group, but not significantly (65% vs. 50%). |

**RCT:** Randomized Controlled Trial; **CBT:** Cognitive Behavioural Therapy; **BDP:** Brief Dynamic Psychotherapy; **SSRI:** Selective Serotonin Reuptake Inhibitor
1.1.4. Prevention Strategies for Postpartum Depression

Given the high prevalence and overall impact of PPD, prevention is an important issue. Presently, several approaches are available for preventing PPD in high risk cases, such as women with a past history of MDE, which may include psychotherapy (IPT/CBT), peer support, the use of antidepressants such as SSRI (Clatworthy 2012; Howard et al. 2005; Wisner et al. 2004) as well as lithium for women with bipolar disorder (Stewart 1988), and dietary supplements such as omega-3s.

The reality is that in the absence of symptoms prior to delivery, pregnant women are preparing for a new child and are usually not interested in obtaining 12 to 18 one hour sessions of therapy needed for IPT or CBT or taking medication for a condition that has not yet manifested. The results of a recent meta-analysis looking at randomized controlled trials (RCT) investigating dietary supplements is preventing PPD, has not found any evidence in their effectiveness (Miller et al. 2013). Therefore, the need for a new method to prevent PPD has been recognized and a recent study investigated interpersonal peer support via phone calls to prevent PPD but the actual rate of PPD at the time-point assessed in enrollees was virtually the same in the group receiving the peer support as the control condition (Dennis et al. 2009). Hence there is still a real need to develop prevention strategies prior to onset of symptoms that are highly feasible (Weissman et al. 2004).

The use of SSRI such as sertraline is the most supported biological intervention, but the two main limitations is that there is not complete accord across the main double blind, RCT and there is also the potential problem of breastfeeding while taking antidepressants. Sertraline was superior to placebo in one randomized clinical trial of prevention of PPD in high risk
women with at least one past episode of PPD (Wisner et al. 2004) although in a subsequent clinical trial it did not differ from the comparator nortriptyline (Wisner et al. 2006). Nortriptyline did not previously distinguish from placebo in a double blind RCT for prevention of recurrent postpartum onset major depression in high risk women (Wisner et al. 2001). However, larger scale RCTs will be desirable, as these previous studies might be under powered due to relatively small number of subjects.

A challenge with SSRI treatment is the issue of breastfeeding, and the present practice is to breastfeed despite maternal intake of antidepressants. The logic is that some of these antidepressants such as sertraline or paroxetine typically have very low levels in babies who are breastfeeding from mothers taking these medications (Misri et al. 2000; Weissman et al. 2004). Moreover, in regards to sertraline the levels of norsertraline, a metabolite of sertraline, are detectable in babies who breastfeed from mothers who take sertraline (Weissman et al. 2004). In the case of paroxetine, breast fed infants typically have undetectable levels of paroxetine (Misri et al. 2000) but there are sporadic cases of paroxetine levels being detected which may be due to error or to the issue that paroxetine plasma levels are highly variable in humans (Weissman et al. 2004). The potential problem of even very modest levels of SSRI in the plasma of infants is that even very low levels of SSRI have a fairly substantial occupancy at the serotonin transporter. For example, the proportion of serotonin transporters blocked at 1/10 the plasma level of regular dosing is approximately 50% (Meyer et al. 2004). Long term data regarding effects of SSRI exposure during breastfeeding is scant. As mentioned, all antidepressants have been shown to pass to breast milk in different extents. So infants are exposed to some amount of antidepressant upon breast feeding. Another limitation of taking antidepressants in early postpartum is that
a common experience in the clinics shows that women do not wish to take an antidepressant to prevent MDE in early postpartum, even when a history of MDE is present.

However, most pregnant women do not have a history of MDE and there are no standard prevention strategies for women with no previous history of MDE. General psychosocial support in postpartum is associated with reduced level of symptoms as measured with scales such as the Edinburgh Postnatal Depression Scale (EPDS) (Dennis and Dowswell 2013), but evidence that diagnostically confirmed PPD is reduced by such interventions has not been established. Hence it is important to develop new methods to prevent PPD, for women with no history of MDE.

Dietary strategies are an unexplored area of potential for preventing PPD. While other areas of medicine, such as cardiology, have made key advances in prevention by applying dietary approaches, in regard to preventing PPD, there have only been 2 RCT investigations on preventing PPD (Mokhber et al. 2011; Mozurkewich et al. 2013). One RCT investigated the effect of selenium (100 ug per day) versus placebo, from the first trimester until delivery, on preventing PPD in 85 women and found that selenium was associated with not a clinical significant difference among two groups. The group receiving selenium had lower EPDS scores compared to the placebo group by a mean difference of -1.9 (8.8±5.1 versus 10.7±4.4 respectively). While statistically significant, the clinical significance of this magnitude is small. In another RCT the effect of eicosapentaenoic acid (EPA) versus docosahexaenoic acid (DHA) versus placebo, from the first trimester, on preventing PPD was investigated in 118 women and there were no differences among groups on preventing PPD measured by Beck Depression Inventory Scale (BDI) within 6 to 8 weeks postpartum.
Efficacy of progestins, including natural progesterone and synthetic progestogens in preventing PPD has also been evaluated (Lawrie et al. 1998). In a double blind placebo controlled study, administration of norethisterone (a synthetic progestogen) in 108 women within 48 hours of delivery was associated with higher EPDS scores at 6 weeks postpartum.

The effect of high dosage of oral premarin (estrogen) on PPD prophylaxis in an open trial taken immediately after childbirth was evaluated in seven women with histories of puerperal psychosis and four with histories of puerperal MDE (Sichel et al. 1995). The outcome was assessed with clinical interview, using DSM-III checklist. Only 1 woman had a relapse of psychosis during the first week of postpartum. All others remained entirely well and required no treatment with psychotropic medications during the 1 year follow-up period.

In Summary, there is need for a widespread prevention strategy for both women who are at low risk and women who are at high risk, such as those with a history of MDE, due to unlikely compliance to other preventative methods.

1.1.4.1. Antidepressant Exposure to Infants during Breastfeeding

Most mothers are reluctant to take antidepressant while breastfeeding, due to the contemplation of infant’s antidepressant exposure. All antidepressants are transferred into breast milk but with high variability (Gentile 2007; Weissman et al. 2004). Transfer of medications into breast milk has been traditionally assessed by the milk to plasma ratio (M/P) (Gentile 2007; Weissman et al. 2004), which varies among different SSRIs, ranging from very low values to approximately 6 (Weissman et al. 2004). While measurement of
antidepressant level in breast milk is important, plasma level of antidepressants in infants is more directly linked to infant exposure and detectable levels of SSRI are often found in infant plasma. At the time these latter studies were conducted, SERT occupancy studies of low dose SSRI had not been completed in humans, so low plasma levels of SSRIs were interpreted as being equivalent to low SERT occupancy a perspective that has not reassessed to date in the context of in vivo SSRI occupancy investigations.

While it is not possible to ethically evaluate occupancy in infants, the relationship between SSRI plasma level and SERT occupancy is known in human adults and low levels of plasma SSRI are associated with substantial brain occupancy due to the non-linear nature of the plasma to occupancy relationship. Based on the relationship between plasma levels and SERT occupancy for specific antidepressants (Meyer et al. 2001; Meyer et al. 2004; Takano et al. 2006), the percentage of occupancy expected for the range of plasma levels of SSRI reported in infants who were exposed to antidepressants exclusively during breastfeeding can be estimated. Even minimal plasma levels of antidepressants are associated with notable SERT occupancy levels in adult humans, suggesting that the plasma levels reported in infants that have been historically viewed as low correspond to substantial occupancies in brain. For the antidepressants sertraline, paroxetine, fluoxetine and citalopram, depending upon the sample size of investigations, approximately 2% to 15% of infants sampled were at the plasma levels corresponding to brain occupancies between 55% and 90%. Another issue in interpreting these earlier studies is that, for several antidepressants, such as sertraline, paroxetine, venlafaxine, and fluvoxamine, the minimum detectable plasma levels selected correspond to fairly high SERT occupancies (in adult humans), being often greater than 40%. Hence for these latter antidepressants, unless it is demonstrated that 40% occupancy has
negligible neurodevelopmental effects postnatally, it is premature to conclude with certainty that there is negligible impact of brain exposure in the subset of 2% to 15% with higher antidepressant plasma levels. The limitation of this approach is the assumption that the plasma:brain ratio of antidepressant medications is similar in infants and adults but, the adult human data is the most valid human information available from which to estimate brain occupancy.

1.2. Proposed Solution, Rationale for Countering Monoamine Oxidase-A Levels in Early Postpartum, A proposed Strategy for Preventing Postpartum Depression

The idea of developing a dietary supplement for preventing PPD is to have a compound that can create resilience and counter biological changes in early postpartum that can lead to PPD.

1.2.1. Biological Changes Reported in Postpartum Depression

Several etiologies have been proposed for PPD and it is likely that the cause is multifactorial. Nonetheless, if several changes are required for PPD to occur, if one can create resiliency against a key implicated mechanism, this might substantially reduce the likelihood of developing PPD.
1.2.1.1. Hormonal Changes

One of the most prominent features of postpartum is hormonal changes. A hormonal origin for PPD has long been theorized (Nott et al. 1976). With tremendous variations in steroid hormones such as progesterone and estradiol and cortisol, it is plausible to consider these changes important in producing depressive like symptoms in postpartum. Hormonal theories of PPD consider striking estradiol and progesterone withdrawal as the main underlying causes of mood symptoms in most women during postpartum (O'Hara et al. 1991b; Workman et al. 2012). However, all women go through these fluctuations during pregnancy and delivery, but only a subset will develop PPD. In addition, there is minimal data still showing that levels of steroid hormones including estrogen, progesterone or cortisol being significantly different among women who develop PPD and those who do not. Some suggest that hormonal variations may contribute more to PPD in women vulnerable to PPD such as those with a history of major depressive episode (Bloch et al. 2003; Bloch et al. 2000; Workman et al. 2012). In a placebo controlled study, high doses of estradiol treatment has been shown to have benefit in treating PPD (Gregoire et al. 1996), however there are need for more RCTs in this area. Synthetic progesterone administration after delivery was associated with higher risk of developing PPD (Lawrie et al. 2000; Lawrie et al. 1998). There have been no RCTs yet on the effect of natural progesterone in the prevention and/or treatment of PPD. Although hormonal alterations have been implicated, the magnitude of these changes is not associated with developing PPD. This may suggest that hormonal changes may lead to a secondary biological change which then contributes to developing PPD. These secondary
biological changes are not necessarily dependant on the magnitude of hormone change (see following sections).

Hypothalamic–Pituitary–Adrenal axis (HPA axis) dysregulation and notably abnormal cortisol levels have been one of the main etiologies of MDD (Parker et al. 2003). Maternal HPA axis undergoes significant changes during pregnancy due to the introduction of placental corticotropin-releasing-hormone (CRH) (Mastorakos and Ilias 2000). This results in significant elevation in CRH, adrenocorticotropin hormone (ACTH) and cortisol concentrations (Mastorakos and Ilias 2000). After delivery, with the loss of placenta, there is a dramatic drop of in CRH levels, and blunted HPA axis activity, normalizing by about 12 weeks postpartum. It has been suggested that in some women HPA axis is not sufficiently suppressed and that this may explain the occurrence of PPD. Among women with PPD, researchers have found a continued blunted response to CRH at 6–12 wk postpartum (Magiakou et al. 1996).

1.2.1.2. Imaging Discoveries

There are several imaging studies of PPD. All have included women with previous MDEs which creates a limitation of being unable to differentiate the effect of a past MDE versus a new effect of PPD. Results from magnetic resonance spectroscopy in 9 PPD women, 14 postpartum healthy and 10 healthy follicular phase women, have shown reductions in occipital cortex Gamma-Aminobutyric Acid (GABA) during postpartum irrespective of having PPD (Epperson et al. 2006). These data suggest that the postpartum period is
associated with reduction in central GABA. GABA levels are reduced during MDE (Luscher et al. 2011).

Data from a positron emission tomography (PET) imaging of postpartum and non-postpartum women has shown reduced D_{2/3} receptor binding potential in the ventral striatum in postpartum, also irrespective of PPD (Moses-Kolko et al. 2012). Subjects included in this study were 13 non-postpartum healthy, 13 postpartum healthy, 10 non-postpartum unipolar depressed, 7 non-postpartum bipolar depressed, 13 postpartum unipolar and 7 postpartum bipolar depressed. All depressed subjects had subclinical to clinical MDE and mean Hamilton rating Scale for Depression scale (HAMD) on the 25 item scale was 19.8±7.6 and 20.1±7.5 for unipolar and bipolar respectively in the past month (All depressed subjects met DSM-IV criteria for a current MDE and had a HAM_{17}≥14 or HAM_{25}≥18 in the past month). When the unipolar MDE and PPD samples were combined, lower D_{2/3} receptor binding potential was observed as compared to healthy controls in ventral striatum (7%–8%) (Moses-Kolko et al. 2012).

In addition, about 20% to 28% reduction has been reported in postsynaptic 5-HT_{1A} receptor binding in the orbitofrontal, subgenual and mesiotemporal cortex in a sample of unipolar and bipolar PPD (Moses-Kolko et al. 2008). This finding implies that lower 5-HT_{1A} receptor binding in MDE also applies to MDE within the postpartum period. This study included 7 postpartum healthy and nine PPD subjects. It should also be noted that most of the subjects with PPD had their onset within pregnancy. The results may suggest that lower postsynaptic 5-HT_{1A} receptor binding might be a general marker of MDE and not only for PPD.

The impression from these studies is that reduced GABA and reduced postsynaptic 5-HT_{1A} receptor binding might be general markers of vulnerability to MDE but further work is
needed to assess the relationship specifically to first onset PPD so as to differentiate the effect of past MDE versus new onset PPD.

Changes in monoamine oxidase-A (MAO-A) will be the basis of this thesis and the timing and magnitude of its changes in postpartum will be discussed extensively in the next sections.

1.2.1.3. Plasma Markers

There have also been changes in a number of blood markers during PPD. Approximately 7% of all new mothers experience thyroid dysfunction after delivery compared to 3% in general population. Some studies have suggested thyroid dysfunction in the etiology of PPD, although there are contrary studies. Thyroid function undergoes changes during pregnancy, including increased thyroid-binding globulin (TBG) (secondary to high estrogen levels), increased total triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}) levels (despite usually normal levels of free T\textsubscript{3} and free T\textsubscript{4} and thyroid stimulating hormone) (Basraon and Costantine 2011; Glinoer 1997). PPD has been associated with an increase in thyroid dysfunction and presence of thyroid antibodies after delivery (Harris et al. 1989; Harris et al. 1992). It should be noted that only in a small subgroup of women thyroid dysfunction was associated to PPD, therefore, other factors seem to play a more etiological role.

As Brain-derived neurotrophic factor (BDNF) is involved in neurogenesis and neuronal plasticity, and therefore regulating synaptic composition, neurotransmitter release and excitability in the nervous system its role in mental health has been studied as well. Low levels of BDNF have been reported in women with PPD compare to postpartum healthy
women (Gazal et al. 2011). Moreover, variations in BDNF have been shown to be associated with hormonal changes in humans (Lommatzsch et al. 2005; Pluchino et al. 2009).

During pregnancy maternal immune system undergoes remarkable change in order to allow the fetus to thrive, while at the same time being able to sustain its own ability to defend against pathogens. This is thought to be done by down-regulation of proinflammatory cytokines (T-cell mediated) and up-regulation of anti-inflammatory cytokines (generalized inflammatory response such as monocytes). After delivery the pro-inflammatory response is significantly elevated and normally after few weeks, as a mother is recovering, the immune system regresses as well. Higher levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, have been reported in women suffering from PPD and MDD (Boufidou et al. 2009; Dowlati et al. 2010). In addition, some suggest PPD as a psychoneuroimmunological disorder as immune system and HPA axis plays a major role and are interconnected.

1.2.1.4. Animal Models of PPD

Several studies have used animal model to address the behavioral changes during postpartum through the hormone withdrawal theory.

In two separate studies by Galea et al in 2001 and Stoffel in 2004, the effect of withdrawal from pregnancy levels of gonadal steroid hormones was evaluated in ovariectomized female rats that underwent a hormone-simulated pregnancy (HSP) regimen (Galea et al. 2001; Stoffel and Craft 2004). The results showed an increased immobility in the forced swim test 2 and 3 days after the last HSP injection, a behavioral profile indicative of MDD. These
studies show that estradiol withdrawal can result in depressive like behavior in ovariectomized HSP animal. However, the applicability of both of these studies to human PPD remains uncertain as the hormone profile associated with pregnancy in rodents in these 2 studies is significantly different from human PPD. As in humans there is withdrawal from both progesterone and estradiol during postpartum as within these 2 studies there was only withdrawal from estradiol.

Suda et al. created a model more consistent with human postpartum (Suda et al. 2008). The ovarian steroids were administered at the levels that occur during human pregnancy and were withdrawal in the same manner. Behavioral testing performed showed that the HSP treatment resulted in the development of a phenotype relevant to PPD, including vulnerability for helplessness, increased anxiety, and aggression. This study pointed out to candidate genes such as BDNF and serotonin transporter (SERT), GABA type A receptor α4 subunit that might underlie these behavioral effects (Suda et al. 2008).

Hippocampal neurogenesis, including cell proliferation and survival has been shown to decrease during postpartum due to fluctuations in gonadal hormones (Galea 2008).

Abrupt decrease in progesterone has been shown to enhance transcription of the gene encoding for GABA type A receptors α4 subunit and induces anxiety (Smith et al. 1998). Of note, the GABA type A receptors δ subunit may be particularly involved in a depressive-like phenotype in lactation, as this receptor subunit undergoes substantial plasticity in the peripartum period. Mice lacking the receptor only display depressive-like behaviour, in both the forced swim test and sucrose preference test, during the postpartum period suggesting a specific temporal association between this receptor subtype and PPD. Interestingly, these mice also display poor maternal care (Maguire and Mody 2008; 2009).
1.2.2. Monoamine Theory of Major Depressive Disorder

Present conceptualization of MDD is that it is a heterogeneous illness with multiple phenotypes, of which one includes processes which reduce monoamines. The monoamine theory of MDD stipulates that depressive symptoms are related to decreased levels of centrally available monoamine neurotransmitters, serotonin, dopamine and norepinephrine (Ruhe et al. 2007). This theory originated from the observations that compounds which decreased the availability of these neurotransmitters, by functions were associated with depressed mood, and has been bolstered by detection of elevated MAO-A levels during MDEs episodes (Chiuccariello et al. 2014; Delgado et al. 1993; Freis 1954; Johnson et al. 2011; Leyton et al. 1997; Leyton et al. 2000; Meyer et al. 2006; Meyer et al. 2009; Young et al. 1985) (detailed further in the following section).

Serotonin, dopamine and norepinephrine can be experimentally depleted in humans. Serotonin synthesis can be reduced by acute tryptophan depletion (ATD) which is performed by ingesting a mixture of all other large neutral amino acids (LNAA) except tryptophan. ATD has shown to cause depressive symptoms, particularly in individuals with family history of MDD or cause relapse in those who have been successfully treated with an SSRI (Delgado et al. 1994; Leyton et al. 1997; Neumeister et al. 2004). Pharmacological blockade of serotonin synthesis by the tryptophan hydroxylase inhibitor p-chlorophenylalanine has been found to reverse the antidepressant effects of monoamine oxidase inhibitor tranylcypromine (Shopsin et al. 1975). Acute phenylalanine tyrosine depletion (APTD) through ingesting a mixture lacking phenylalanine and tyrosine, has been shown to decrease dopamine and norepinephrine synthesis and has been associated with inducing depressed
mood (Leyton et al. 2003). Similarly, tyrosine hydroxylase, a rate limiting enzyme in catecholamine synthesis, can be blocked with α-methyl-para-tyrosine (AMPT) (Delgado et al. 1993). Administration of AMPT itself may induce sad mood in healthy subjects (Laruelle et al. 1997; Verhoeff et al. 2003). Also, treatment with AMPT can induce relapse in patients who have been treated successfully with a norepinephrine reuptake inhibitor, but not in healthy subjects (Ruhe et al. 2007).

Nevertheless, studies performed to date have shown that acute decrease in serotonin, dopamine and norepinephrine synthesis or all combined does not cause depressive symptoms in healthy individuals with no family history of MDD. These findings suggest that serotonin levels above a certain threshold are essential for antidepressant efficacy of SSRI. It also implies that acute monoamine depletion is not sufficient to induce depressive symptoms in otherwise healthy individuals.

1.2.3. What is Monoamine Oxidase-A

As mentioned, the etiology of PPD is likely multifactorial as social and few biological factors have been identified, but if one can prevent an important factor in early postpartum, this should reduce the risk of PPD. In this thesis we will explain why elevated MAO-A levels early in postpartum is strongly implicated as an important factor in the onset of PPD. MAO-A is an enzyme related to many mood disorders and also involved in the mechanisms of abnormality in PPD. Subsequently, we make a case for developing a dietary supplement to counter the effects of elevated MAO-A levels to prevent PPD.
Monoamine Oxidase A is an important enzyme found on the outer mitochondrial membrane in neurons and glia in the brain (Saura et al. 1996; Westlund et al. 1993; Youdim et al. 2006). In human brain MAO-A density is homogenously distributed within in most brain structures including cortex, with the highest density in the locus coeruleus, and high density in cortex, striatum, thalamus, with lower density in cerebellar cortex and the lowest density in white matter (Ginovart et al. 2006; Saura et al. 1996; Saura et al. 1992). MAO-A has a key role since it metabolizes serotonin, norepinephrine and dopamine; increases oxidative state; and influences predisposition to apoptosis (Ou et al. 2006; Youdim et al. 2006). MAO-A density is highly correlated with its function (Nelson et al. 1979b; Saura et al. 1992). Hence elevation of MAO-A levels would be expected to influence mood through lowering these monoamines, given that paradigms that deplete these monoamines such as tryptophan depletion, inhibition of tyrosine hydroxylase or breakdown of the storage of these monoamines, is associated with low mood (Delgado et al. 1993; Freis 1954; Hasler et al. 2008; Leyton et al. 1997; Leyton et al. 2000; Young et al. 1985).

Monoamine Oxidase-A catalyses the oxidative deamination of serotonin, dopamine, and norepinephrine (Ou et al. 2006; Youdim et al. 2006). One of the products of this reaction is hydrogen peroxide ($H_2O_2$). At low to moderate levels, $H_2O_2$ is considered physiological and is necessary for some physiological processes in the cell, such as response to trauma or inflammation. However with higher MAO-A activity, these levels might be supraphysiological and may generate excessive oxidative stress and have potential adverse cellular consequences (Villeneuve et al. 2013). Oxidative stress has been shown to be associated with anxiety behaviours in rodents and markers of oxidative stress are sometimes elevated in mood disorders (Bortolato et al. 2008; Bouayed et al. 2009; Brown et al. 2014;
In one study the use of vitamin C as an antioxidant in mice that were under stress prevented the increase in MAO-A mRNA (Lee et al. 2012).

Evidence that MAO-A metabolizes serotonin, norepinephrine and dopamine is described further below:

1.2.3.1. Relationship of MAO-A to Extracellular Serotonin

Serotonin is a high affinity substrate for MAO-A (Fowler and Oreland 1979; Kinemuchi et al. 1984; Schoepp and Azzaro 1981; White and Tansik 1979) and MAO-A is detectable in serotonin releasing neurons (Konradi et al. 1988; Luque et al. 1995). MAO-A clearly influences extracellular serotonin because administration of MAO-A inhibitors increases extracellular serotonin from 20% to 200%, depending upon drug, dose and region (Adell et al. 1996; Bel and Artigas 1995; Celada and Artigas 1993; Curet et al. 1998; Fagervall and Ross 1986; Haefely et al. 1992). This has been found in at least six separate studies and across four different MAO-A inhibitors (clorgyline, moclobemide, harman, befloxatone) (Adell et al. 1996; Bel and Artigas 1995; Celada and Artigas 1993; Curet et al. 1998; Haefely et al. 1992) and the finding was present in a variety of brain regions including prefrontal cortex, hippocampus, and superior raphe nuclei. In these paradigms it is often demonstrated that brain 5-HIAA is reduced (Adell et al. 1996; Bel and Artigas 1995; Curet et al. 1998). Moreover, extracellular serotonin is also raised substantively (100% to 200%) in prefrontal cortex, hippocampus and superior raphe nuclei in the knockout model of MAO-A (Evrard et al. 2002).
1.2.3.2. Relationship of MAO-A to Norepinephrine

Norepinephrine is a high affinity substrate for MAO-A (Houslay and Tipton 1974; White and Tansik 1979) and MAO-A is easily detectable in cells that synthesize norepinephrine (Konradi et al. 1989; Konradi et al. 1988; Luque et al. 1995; Saura et al. 1996). Under conditions of MAO-A inhibition, extracellular norepinephrine is increased in prefrontal cortex as well as hippocampus (regions assayed in the studies) (Fagervall and Ross 1986; Finberg et al. 1994; Finberg et al. 1993) which argues that MAO-A has a substantial role in controlling extracellular norepinephrine.

1.2.3.3. Relationship of MAO-A to Dopamine

Dopamine is also high affinity substrate for MAO-A (Fowler and Oreland 1979; Kinemuchi et al. 1984; Schoepp and Azzaro 1981) and administration of MAO-A inhibitors increases extracellular dopamine in striatum under baseline conditions as well as during precursor loading paradigms (Adachi et al. 2001; Brannan et al. 1995; Butcher et al. 1990; Colzi et al. 1992; Colzi et al. 1990; Finberg et al. 1995; Segal et al. 1992; Wayment et al. 2001).
1.2.4. Monoamine Oxidase-A and its Relationship to Major Depressive Disorder

There is substantial amount of data connecting loss of extracellular neurotransmitters serotonin, dopamine and norepinephrine in humans with onset of MDD and MDEs. Therefore, it would be plausible that MAO-A elevation in affect modulating regions will manipulate the mood towards sad mood and depressive symptoms. In a PET study done in 2006, MAO-A DV$_S$, an index of MAO-A density, was measured in medication free MDE (secondary to early onset MDD) and healthy subjects. MAO-A was significantly elevated by 34% in prefrontal and anterior cingulate cortex in depressed subjects ($p<0.001$ each region) as compared to controls (Meyer et al. 2006) (Figure 1-1). This finding has been since replicated in 2009 in a separate sample by the same laboratory and also replicated by a different laboratory in 2011 in antidepressant free MDE subjects in postmortem study of orbitofrontal cortex applying western blot (Johnson et al. 2011; Meyer et al. 2009).

Elevated brain MAO-A density during MDEs has imperative implications for the monoamine theory of depression. An advanced monoamine theory can be understood as detailed in Figure 1-2).
Figure 1-1. Greater Monoamine Oxidase A (MAO-A) Distribution Volume, an Index of MAO-A Levels, During Major Depressive Episodes

Comparison of Monoamine Oxidase-A Specific Distribution Volume (MAO-A DVs) between Depressed and Healthy Subjects. On Average MAO-A DVs was Elevated by 34% in Depressed Individuals (*p=0.001, **p<0.0001, ***p<0.00001) (Meyer et al. 2006)

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Elevations in MAO-A $V_T$, an index of MAO-A density, is also observed in the prefrontal and anterior cingulate cortex during other sad/dysphoric states such as early alcohol withdrawal and early cigarette withdrawal. People with alcohol dependence regularly experience sadness and dysphoria during early alcohol withdrawal (APA 2000; Bokstrom et al. 1989). In a recent study using PET in subjects with alcohol withdrawal it was discovered that MAO-A $V_T$ was elevated by 35% in the prefrontal and anterior cingulate cortex. MAO-A $V_T$ in these regions was correlated with severity of depressed mood (Matthews et al. 2014). In addition people who smoke cigarettes also regularly experience sadness and dysphoria during acute cigarette withdrawal (Carey et al. 1993; Kenford et al. 2002) and in another study using PET, MAO-A $V_T$ after early cigarette withdrawal from heavy cigarette smoking was compared to the active smoking condition. During withdrawal, MAO-A $V_T$ was elevated by 25% in the prefrontal and anterior cingulate cortex (Bacher et al. 2011). Also, the magnitude of rise in MAO-A $V_T$ in these regions was significantly correlated with the shift in the mood scores on visual analogue scales (VAS) towards depressed mood (Bacher et al. 2011).

Moreover, recovered state of MDD is a state of high risk for another MDE. Results from another PET study in recovered MDD subjects compared to healthy controls, has shown that MAO-A $V_T$ is significantly elevated in the prefrontal and anterior cingulate cortex of recovered subjects (Meyer et al. 2009). The highest levels were observed in recovered MDD who had recurrence of their MDE in the following 6 months (Meyer et al. 2009).
Figure 1-2. Modernization of Monoamine Theory of Depression

(A), Description of monoamine release in a synapse in a healthy person. (B), During a major depressive episode (MDE), monoamine oxidase A (MAO-A) density is elevated, resulting in greater metabolism of monoamines, such as serotonin, norepinephrine, and dopamine, in the brain. C and D, Range of outcomes. If the monoamine transporter density for a particular monoamine is low during MDE (C), the effect of an elevated MAO-A level on reducing that particular monoamine in the extracellular space is somewhat attenuated, resulting in a moderate loss of monoamine. This eventually results in a moderate severity of symptoms associated with long-term loss of that particular monoamine. If the monoamine transporter density for a particular monoamine is not low during a MDE (D), then there is no protection against the effect of elevated MAO-A levels. The extracellular concentration of the monoamine is severely reduced, and symptoms associated with long-term loss of that particular monoamine eventually become severe. Some postsynaptic receptors increase in density when their endogenous monoamine level is low in the long term. (Meyer et al. 2006)
1.2.5. Monoamine Oxidase-A and its Relationship to Postpartum Mood Symptoms

During pregnancy, estradiol and estriol are produced by the placenta and plasma levels rise 100 fold and 1000 fold respectively (Hendrick et al. 1998). After delivery, with the loss of placenta, these levels drop abruptly with most of the decline occurring in the first three days with a modest decline thereafter (Nott et al. 1976; O'Hara et al. 1991a; O'Hara et al. 1991b). There is an inverse relationship between changes in estrogen and changes in MAO-A density, synthesis and activity in cell lines, in regions of high MAO-A density in rodents (amygdala, cortex), and regions of high MAO-A density in Macaque monkeys (dorsal raphe nucleus) (Gundlah et al. 2002; Holschneider et al. 1998; Leung et al. 1980; Luine and McEwen 1977; Ma et al. 1993; Ma et al. 1995; Smith et al. 2004). In a recent study in humans using PET in early postpartum as compared to not recently pregnant women, it was discovered that MAO-A $V_T$ was significantly elevated by 43% during days 4 to 6 postpartum (Sacher et al. 2010) (Figure 1-3). Consequently, the acute rise in MAO-A $V_T$ in the early postpartum period characterizes a monoamine-lowering process, which can be related to sad mood (Ruhe et al. 2007). This is observed as a new neurobiological explanation of postpartum blues and a phenomenon that predisposes to postpartum blues and PPD (Figure 1-4).
Figure 1-3. Greater Monoamine Oxidase A (MAO-A) Binding in Immediate Postpartum Period

Monoamine Oxidase-A Total Distribution Volume Values were, on Average Elevated by 43% in Early Postpartum as Compared to Women Not Recently Pregnant throughout All Analyzed Brain Regions (Sacher et al. 2010)

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Monoamine model of postpartum blues (which its severity is associated with increased risk of postpartum depression). After delivery, estrogen levels drop 100- to 1000- fold; the estrogen decline is greatest during the first 3 to 4 days postpartum, with a modest decline thereafter. Monoamine Oxidase-A (MAO-A) levels are significantly greater in early postpartum period, with a peak on day 5 postpartum. In the early postpartum period, up to 75% of women experience sadness, mood lability, anxiety, insomnia, poor appetite, and irritability, with mood being lowest on day 5 postpartum (Sacher et al. 2010).
1.2.6. Proposed Solution

Although there are different etiologies implicated in PPD, the focus of this project is on compensating for the elevated MAO-A level in early postpartum. There are a number of compelling reasons for this:

1) Greater MAO-A $V_T$ is the biggest magnitude of change in early postpartum reported to date (Sacher et al. 2010)

2) Elevation in MAO-A level in early postpartum has been demonstrated in human (Sacher et al. 2010)

3) This marker has a strong relationship with the onset of major depressive episodes (Meyer et al. 2006)

4) The peak effect of MAO-A elevation occurs during postpartum blues and it is well known that the severity of postpartum blues is associated with PPD (O'Hara et al. 1991b; Sacher et al. 2010)

5) There is a logical strategy that could address this change in postpartum (Described below)

The proposed solution is to counter the effects of high MAO-A in a time that high metabolizing MAO-A process in postpartum is observed. This may be done by providing monoamine precursors through supplementation of amino acids tryptophan and tyrosine which become neurotransmitters serotonin, norepinephrine and dopamine as well as adding antioxidants from blueberry juice with blueberry extract. It is known that none of these monoamine neurotransmitters directly cross the blood brain barrier, rather, precursor amino acids cross the blood brain barrier through a transport mechanism (Young and Leyton 2002).
Tryptophan is an essential dietary amino acid and is the precursor for serotonin. Tyrosine is a non-essential dietary amino acid and is the precursor for norepinephrine and dopamine (Leyton et al. 2000; Young and Leyton 2002). Both of these amino acids are transported via a carrier process that has a preference for large neutral amino acids (LNAA) (Hawkins et al. 2006).

Tryptophan is found in many foods. For example there is 300mg per 100g of turkey and 30mg per 100g of dried beans (Comai et al. 2007; Holden 2007). The principal role of tryptophan in human is protein synthesis. In the brain, tryptophan is converted by tryptophan hydroxylase into 5-hydroxytryptophan and then into serotonin via amino acid decarboxylase (Cooper et al. 1991; Fernstrom 1981). Serotonin does not directly cross the blood brain barrier but tryptophan does (Koskiniemi et al. 1985; Van Praag 1990; Young 1996). Relative depletions of tryptophan have been shown to lower mood in humans (Benkelfat et al. 1994; Oldman et al. 1994; Smith et al. 1997; Young et al. 1985). L-tryptophan is available in Canada in health food stores at doses below 220mg; however, it requires prescription in oral dosage form at a concentration of more than 220mg per dosage unit or per daily dose, as a single ingredient or in combination with other ingredients.

Tyrosine is found in many foods as well, for example, there is 180 mg of tyrosine per 100g of whole cow milk and 725mg of tyrosine per 100g of chicken meat (Owasoyo et al. 1992). Tyrosine is hydroxylated by tyrosine hydroxylase into DOPA in the brain, which is then converted into dopamine by the enzyme dopa decarboxylase (Fernstrom 1981). In neurons that store and release norepinephrine, this neurotransmitter is formed by betahydroxylation of dopamine (Fernstrom 1981). Depletion of this precursor may lower mood (Leyton et al. 2000). Tyrosine is available as a supplement in 500mg amounts.
Combination of L-Tryptophan and tyrosine has not previously been evaluated for prophylaxis against MDE or PPD. There has been one clinical trial in which tryptophan and tyrosine were combined as a treatment: 100mg/kg per day of tyrosine or placebo was added to 5g per day of l-tryptophan treatment in ten depressed subjects (Van Praag 1990). The combined treatment resulted in a final average Hamilton Depression Rating Scale (HDRS) of 9 whereas the tryptophan with placebo resulted in a final average HDRS of 17. Statistical results were not presented but the means were three standard deviations apart. These very preliminary results were interesting as a score of 17 on the HDRS is the minimum score for a MDE, a score of below 8 is considered a remission and a reduction of 50 per cent on the HDRS is considered a response.

Most of the investigations of tyrosine support its ability to protect against adverse cognitive effects of stress: Under conditions of cold stress, noise stress, and hypoxic stress, single doses of 100mg/kg (Banderet and Lieberman 1989; Deijen and Orlebeke 1994), 150mg/kg (Magill et al. 2003; Owasoyo et al. 1992; Shurtleff et al. 1994; Sutton et al. 2005) and 300mg/kg (Mahoney et al. 2007a; O'Brien et al. 2007) were helpful in creating resilience, based upon unimpaired performance upon cognitive tasks, mainly in the area of working memory. The earlier investigations assessed doses of 150mg/kg whereas the later doses assessed 300mg/kg (Mahoney et al. 2007a; O'Brien et al. 2007). These single dosing regimens were extremely well tolerated. A double blind study of 100mg/kg/day of tyrosine to treat MDD in 17 depressed subjects was not significantly different as compared to 17 healthy controls, although it is likely that this study is underpowered (Gelenberg et al. 1990). The treatment was well tolerated: Of the 21 depressed subjects originally enrolled to receive tyrosine, one discontinued due to palpitations (which are also common during anxiety that
often occurs during major depressive episodes). In an earlier study of 14 subjects by the same group, 4 of 6 subjects treated with 100mg/kg of tyrosine, for four weeks, reached remission as compared to 3 of 8 receiving placebo (Gelenberg et al. 1982). Another study of 6 subjects investigated the effects of 9g of tyrosine per day for four weeks for narcolepsy and reported excellent tolerability (Elwes et al. 1989). A report of a double blind crossover trial of 140mg/kg/day in 14 children reported that it was well tolerated (Nemzer et al. 1986).

Tryptophan alone has been evaluated to prevent postpartum blues at a low dose of 3g in a placebo controlled trial (Harris 1980). We think the reason there was no effect was that multiple monoamines need to be raised, rather than a single monoamine. The compliance of this trial might have been low too, as it was not mentioned to have been evaluated. Clinical trials in MDEs show some promise but are inconclusive. Most enrolled samples of less than 40 subjects, and many are not blinded (Broadhurst 1970; Bunney et al. 1971; Carroll et al. 1970; Coppen et al. 1972; Gayford et al. 1973; Kline and Shah 1973). However, most of these studies investigated doses of L-tryptophan between 5 and 10g per day and reported that this was well tolerated, at least during MDE. The most definitive study that we are aware of was conducted by Thomson et al. (Thomson et al. 1982) in which 3g of L-tryptophan per day was superior to placebo and similar to antidepressant treatment. Strengths of the study were that there was an average of 30 subjects assigned to each cell and each subject was antidepressant free for at least 2 weeks. Weaknesses of the study were that one third of the L-tryptophan sample dropped out and half of the placebo group dropped out of the study.

Higher MAO-A levels are associated with an increase in oxidative stress, as MAO-A catalyses the oxidative deamination of monoamine neurotransmitters serotonin, dopamine and norepinephrine (Ou et al. 2006; Youdim and Bakhle 2006). While many foods and fruits
contain antioxidants (Halvorsen et al. 2006), blueberries were chosen for their antioxidant content because they contain a number of different anthocyanins in contrast to other fruits which have mainly cyanidin anthocyanins (Table 1-2) (Wu et al. 2006). A number of the anthocyanins, especially, malvidin and cyanidin anthocyanins have been reported to be detectable in the brains of blueberry fed rodents and pigs, and blueberry administration is associated with resistance to cognitive decline in rodents, suggesting again that the contents are brain penetrant (Andres-Lacueva et al. 2005; Joseph et al. 2003; Joseph et al. 1999; Joseph et al. 1998; Kalt et al. 2008).
Table 1-2. Anthocyanin Concentrations in Common Foods (Wu et al. 2006)

<table>
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<tr>
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<th>Dp-ACN</th>
<th>Cy-ACN</th>
<th>Pt-ACN</th>
<th>Pg-ACN</th>
<th>Pn-ACN</th>
<th>Mv-ACN</th>
<th>Total ACN</th>
<th>Total ACN/ serving² (mg)</th>
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</tbody>
</table>

¹ All data expressed milligrams of anthocyanin on an “as is” weight basis and presented as mean ± SD for sample number>2;
² Serving size from the USDA National Nutrient database for standard reference; ACN: Anthocyanin; Dp: Delphinidin; Cy: Cyanidin; Pt: Petunidin; Pg: Pelargonidin; Pn: Peonidin; Mv: Malvidin
1.2.7. Advantages of Proposed Dietary Supplementation

Prior to evaluating the effect of combination of tryptophan and tyrosine in preventing mood symptoms in postpartum, it is essential to know the effect of these supplements on their concentrations in breast milk of breastfeeding mothers after oral ingestion. There have been no studies so far to point out on the effect of tyrosine and tryptophan on amino acid contents of breast milk.

Many amino acids and most medications freely cross into breast milk. It is known now that all antidepressants cross into breast milk with different extents. In contrast to medications, such crossing should not affect total concentrations of amino acids such as tryptophan and tyrosine into breast milk. The reason is that approximately 98% to 99% of tryptophan and tyrosine in breast milk are contained in proteins and peptides (Lemons et al. 1983; Macy 1949; Svanberg et al. 1977; Wurtman and Fernstrom 1979). While it has never been investigated, given the overwhelming proportion of amino acids in fixed chain sequences of proteins and peptides, it would be expected that oral supplementation of tryptophan and tyrosine should have no effect upon total levels of these amino acids in breast milk. However, it is important to empirically test this, as was done in the early phase of my thesis.

Subsequently, the effect of the supplements on reducing the mood symptoms in postpartum will be evaluated at the highest dose which does not affect its concentration in breast milk. As postpartum blues increases the risk of PPD and the more severe the postpartum blues the higher the risk of PPD, detection and quantification of postpartum blues is of importance. However, the current questionnaires and scales that are used to detect specifically postpartum blues which include Visual Analog Scale (VAS), Brisbane Blues Scale, Stein’s Maternity
Blue Scale and Edinburgh Postnatal Depression Scale (EPDS) (Henshaw 2003) do not fully capture both the state and phenomenology of vulnerability to sad mood during postpartum blues. Prior to investigating the effect of the supplements on reducing mood symptoms in postpartum, we would also like to investigate if Mood Induction Procedure (MIP) has the ability to quantify postpartum blues during days 4 to 6 postpartum and also in mothers who have crying spells in the first year of postpartum. Previous data also indicates that 20% of women have crying spells in their first year of postpartum and this phenomenology is associated with 15% elevation in MAO-A binding throughout the brain (Sacher et al. 2015). The results of this study will assist us to more optimally quantify postpartum blues and help us explore ways to prevent PPD.
1.3. Objectives

The specific objectives of this thesis, demonstrated through 4 separate studies were:

**Chapter 2:** No effect of oral tyrosine on total tyrosine levels of breast milk.

*Objective 1:* To determine whether changes in total tyrosine contents in breast milk will be negligible after oral tyrosine supplements.

*Objective 2:* To determine whether plasma tyrosine levels will increase with magnitude of oral tyrosine supplements.

**Chapter 3:** No effect of oral tryptophan and oral alpha-lactalbumin on total tryptophan levels of breast milk.

*Objective 1:* To determine whether changes in total tryptophan contents in breast milk will be negligible after oral tryptophan or tryptophan containing protein supplements.

*Objective 2:* To determine whether plasma tryptophan levels will increase with magnitude of oral tryptophan or tryptophan containing protein supplements.

**Chapter 4:** Quantitating predisposition to depressed mood in postpartum.

*Objective 1:* To determine whether sadness after mood induction will be higher in day 5 healthy postpartum women compared to healthy non-postpartum women.

*Objective 2:* To determine whether sadness after mood induction will be higher in women with crying spells during first year postpartum as compared to healthy non-postpartum women.
**Chapter 5:** Effect of dietary supplement on predisposition to depressed mood in an open trial in postpartum.

**Objective 1:** To determine whether dietary supplementation with tryptophan, tyrosine and blueberry extract will reduce sadness after sad mood induction in day 5 postpartum women (during the typical peak of postpartum blues) compared to those not receiving any supplements.
1.4. Hypotheses

The specific hypotheses of this thesis, demonstrated through 4 separate studies were:

**Chapter 2:** No effect of oral tyrosine on total tyrosine levels of breast milk.

**Hypothesis 1:** Changes in total tyrosine contents in breast milk will be negligible after oral tyrosine supplements.

**Hypothesis 2:** Plasma tyrosine levels will increase with magnitude of oral tyrosine supplements.

**Chapter 3:** No effect of oral tryptophan and oral alpha-lactalbumin on total tryptophan levels of breast milk.

**Hypothesis 1:** Changes in total tryptophan contents in breast milk will be negligible after oral tryptophan or tryptophan containing protein supplements.

**Hypothesis 2:** Plasma tryptophan levels will increase with magnitude of oral tryptophan or tryptophan containing protein supplements.

**Chapter 4:** Quantitating predisposition to depressed mood in postpartum.

**Hypothesis 1:** Sadness after mood induction will be higher in day 5 postpartum women compared to non-postpartum women.

**Hypothesis 2:** Sadness after mood induction will be higher in women who have crying spells during first 18 months of postpartum compared to the women who do not have crying spells in the first 18 months postpartum.
Chapter 5: Effect of dietary supplement on predisposition to depressed mood in an open trial in postpartum.

Hypothesis 1: Sadness after mood induction in day-5 postpartum women (during the typical peak of postpartum blues) will be attenuated in those receiving the dietary supplement (tryptophan, tyrosine and blueberry juice) compared to those not receiving any supplements.
Chapter 2  No Effect of Oral Tyrosine on Total Tyrosine Levels of Breast Milk

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2.1. Abstract

**Purpose:** Postpartum depression (PPD) is the most common complication of childbearing with a 13% prevalence rate and there is no widespread approach for prevention. There is an appealing theoretical rationale for oral tyrosine to help prevent PPD. However, the effect of oral tyrosine on its total and free concentrations in breast milk and plasma of breastfeeding mothers is not known.

**Methods:** Twenty-four healthy breastfeeding women were randomly assigned to 0, 2, 5, or 10 grams of oral tyrosine. Free and total tyrosine in breast milk and free tyrosine in plasma were measured. Free tyrosine was also measured in 12 different infant formulas.

**Results:** Total tyrosine in breast milk did not rise but there was a slight tendency towards a reduction (up to -12%; Repeated Measures of ANOVA (rANOVA): \( p=0.074 \)). Maternal plasma tyrosine rose (rANOVA: \( p<0.005 \)). In breast milk, 98% of tyrosine was in proteins or peptides and 2% was free. Free tyrosine levels in breast milk rose in each group (rANOVA: \( p<0.005 \)) but levels were within the range found in common infant formulas.

**Conclusion:** The negligible effect of oral tyrosine on its concentration in breast milk supports further development of oral tyrosine as part of a prevention strategy for PPD.
2.2. Introduction

Postpartum depression (PPD) is a frequent, impactful, complication of childbearing, with a prevalence rate of 13% (O'Hara and Swain 1996). One immediate concern of PPD is that it is associated with at least a 10 fold greater risk of suicide attempts (Comtois et al. 2008). Long term outcome of PPD is also problematic because PPD may last for years (Goodman 2004; Horowitz and Goodman 2004) and PPD is associated with greater risk of recurrent major depressive episodes (MDE) both following future deliveries and during periods unrelated to childbirth (Kumar and Robson 1984; Nott 1987; Philipps and O'Hara 1991; Warner et al. 1996; Wisner et al. 2002). In addition, PPD is associated with reduced infant motor and social development, and less optimal health of other children in the family and the partner (NICE clinical guideline 2007; O'Hara 1994).

Given the high prevalence, and overall impact of PPD, prevention is an important issue. Presently, several approaches for preventing of PPD for women with a past history of MDE are available such as interpersonal therapy, cognitive behavioural therapy, and use of antidepressants such as selective serotonin reuptake inhibitors (Dennis and Dowswell 2013; Miller and LaRusso 2011) as well as lithium for women with bipolar disorder (Stewart 1988). However, most pregnant women do not have a history of MDE and there are no standard prevention strategies for women with no previous history of MDE. General psychosocial support in postpartum is associated with reduced level of symptoms as measured with scales such as the Edinburgh Postnatal Depression Scale (Dennis and Dowswell 2013), but evidence that diagnostically confirmed PPD is reduced by such
interventions has not been established. Hence it is important to develop new methods to prevent PPD, for women with no history of MDE.

Dietary strategies are an unexplored area of potential for preventing PPD. While other areas of medicine, such as cardiology, have made key advances in prevention by applying dietary approaches, in regard to preventing PPD, there have only been some preliminary investigations of the benefit of fish oil. These studies to date have not yielded positive results (Amminger et al. 2010), although these investigations may be underpowered. Although a dietary supplement strategy of several key ingredients to prevent PPD is appealing, it is also challenging because ingredients exceeding the limits of the usual dietary intake would require testing to assess its effects upon breast milk as the first step of development.

There are three main reasons why dietary tyrosine may have utility as a component of a dietary prevention strategy to protect against PPD. The first is that MAO-A V_T, an index of MAO-A levels are elevated by over 40% in early postpartum (Sacher et al. 2010). Greater MAO-A levels in early postpartum may contribute to a predisposition to PPD: MAO-A levels are highly correlated with MAO-A activity in brain (Nelson et al. 1979a; Saura et al. 1992). MAO-A has a high density in brain regions implicated in affect regulation and MAO-A oxidatively metabolizes mood regulating chemicals such as serotonin, norepinephrine and dopamine. Greater levels of MAO-A in the prefrontal and anterior cingulate cortex occur during MDEs and postpartum MDEs, as well as during high risk periods for the condition, including prior to recurrence of illness (Bacher et al. 2011; Johnson et al. 2011; Meyer et al. 2006; Meyer et al. 2009; Sacher et al. 2010; Sacher et al. 2011). Given this model of greater predisposition to MDE when high MAO-A levels are present in the prefrontal and anterior
cingulate cortex, tyrosine administration may useful as a means to counter this vulnerability. Tyrosine is the amino acid precursor to norepinephrine and dopamine in the brain, neurotransmitters metabolized by MAO-A. Norepinephrine and dopamine do not cross the blood-brain barrier directly and are dependent upon precursor transport. Tyrosine, being a large neutral amino acid (LNAA), is transported into the brain through a LNAA transporter system, which it shares and competes for with other LNAAs (Pardridge 1977). Therefore, tyrosine quantity and availability to the brain may influence the synthesis rate and brain levels of norepinephrine and dopamine, two of the neurochemicals metabolized by MAO-A (Growdon et al. 1982; Van Praag 1990; Young 1996).

The second reason for developing oral tyrosine as a dietary strategy to protect against PPD is its ability to enhance resilience against stress. Stressors evaluated include cold exposure and noise stress and these two conditions are associated with depressive behaviors in rodents (Lapiz-Bluhm et al. 2009; Naqvi et al.). In humans, at oral tyrosine doses of 100-300mg/kg resilience to such stressors was demonstrated based upon unimpaired performance on cognitive tasks, mainly in the area of working memory, short-term memory, psychomotor performance, mainly evaluated by marksmanship, and a lesser tendency towards negative mood state (Banderet and Lieberman 1989; Deijen and Orlebeke 1994; Mahoney et al. 2007b; O'Brien et al. 2007; Shurtleff et al. 1994).

The third reason for developing oral tyrosine as part of a dietary strategy to prevent PPD is that there is a good theoretical rationale why its oral administration might not affect its levels in breast milk. There are three pools of tyrosine in breast milk as tyrosine is contained in proteins, peptides and as free tyrosine. The proportion of tyrosine in the first two pools is fixed because the proteins and peptides have fixed ratios of amino acids and 99% of tyrosine
in breast milk is contained in these two pools (Lemons et al. 1983; Svanberg et al. 1977; Wurtman and Fernstrom 1979). While it would be expected that free tyrosine would not have this restriction upon its ratio relative to other amino acids, its proportion is very small. However, it should be noted that to the best of our knowledge, the effect of oral tyrosine (or any amino acid) on its levels (either total or free) in breast milk has not been previously investigated.

The main objective of this study was to evaluate the effects of acute oral tyrosine supplementation on its total levels in breast milk. It was hypothesized that the total levels of tyrosine in breast milk will not change significantly at doses that would normally raise tyrosine levels in plasma in humans. Our second objective was to assess the effects of acute oral tyrosine supplementation on free tyrosine levels in breast milk as this also has not been previously studied. It was hypothesized that free tyrosine levels in breast milk, which are not restricted like other pools of tyrosine in breast milk, will be elevated. To establish what range of exposures to free tyrosine in breast milk have been approved as safe, our third objective is to determine tyrosine levels across 12 different infant formulas that are commonly used in North America and Europe. To our knowledge, this last question has not been studied in a sufficiently large dataset to compare statistically since the largest investigation sampled only 1 to 2 bottles of formula across a few brands (Agostoni et al. 2000b; Alegria et al. 1999; Ventura et al. 2012).
2.3. Methods

Women aged 18 to 40 were recruited through advertisement from the Greater Toronto Area, Ontario, Canada. They were eligible to participate if they were healthy, currently breastfeeding within the second to 24th months postpartum, medication-free and not taking over the counter medication. Exclusion criteria were: currently pregnant (as screened with urine pregnancy test), use of any investigational medicinal product or herbal medication within the previous 8 weeks, self report of current psychiatric or medical illnesses, suicide attempts, substance abuse (screened with urine drug test), known hypersensitivity to tyrosine, and history of severe drug allergy or drug hypersensitivity involving an anaphylactic reaction.

Women were randomly assigned to one of four groups to receive a single oral dose of 2, 5 or 10 g of tyrosine or the control group without tyrosine supplements. Tyrosine capsules were obtained from Trophic Canada and each capsule contained 500 milligrams of l-tyrosine (approved by Health Canada) (Trophic Canada, Richmond Hill, Ontario, Canada, NPN#80010004). The Trophic l-tyrosine has been approved for use by Health Canada based on purity, consistency of content and lack of toxins. The product monograph of this product can be found at Health Canada’s website.

Subjects first came for a screening visit to be evaluated for eligibility. For the second visit, subjects attended the laboratory at 8:00 am and underwent procedures described in Table 2-1. Dietary intake was standardized for all subjects to enhance the ability to detect the effect of the different doses of tyrosine supplements. To standardize their diet, subjects were asked to fast from 10 pm the night before. During their second visit all subjects were provided with
a standardized protein-free breakfast and lunch. Four breast milk samples (one prior to supplement ingestion and the remaining 3 over the subsequent hours), and 7 blood samples (two prior to supplement ingestion and the remaining 5 over the subsequent hours) were collected at different time points. Thirty milliliters of breast milk at each time point were obtained with an electric pump (Medela Symphony Breast pump), transferred into sterile polypropylene vials, coded and frozen at -80°C after collection until assay. Ten milliliters of maternal blood samples at each time point were obtained by performing a onetime venipuncture to establish a saline lock followed by sampling from the lock at each time point. During the main study day, infants were bottle fed until after the last sample was taken.

Table 2-1. Overview of the Second Visit

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 am</td>
<td>Arrival</td>
</tr>
<tr>
<td>8:15 am</td>
<td>Plasma Sample</td>
</tr>
<tr>
<td>8:30 am</td>
<td>Plasma Sample / Breast Milk Sample</td>
</tr>
<tr>
<td>8:40 am</td>
<td>Tyrosine Intake</td>
</tr>
<tr>
<td>9:00 am</td>
<td>Low Protein Breakfast</td>
</tr>
<tr>
<td>9:30 am</td>
<td>Plasma Sample</td>
</tr>
<tr>
<td>10:30 am</td>
<td>Plasma Sample / Breast Milk Sample</td>
</tr>
<tr>
<td>11:30 am</td>
<td>Plasma Sample</td>
</tr>
<tr>
<td>12:00 pm</td>
<td>Low Protein Lunch</td>
</tr>
<tr>
<td>12:30 pm</td>
<td>Plasma Sample / Breast Milk Sample</td>
</tr>
<tr>
<td>2:30 pm</td>
<td>Plasma Sample / Breast Milk Sample</td>
</tr>
</tbody>
</table>
Levels of free tyrosine in 12 infant formulas were also analyzed in order to compare amino acid levels in breast milk with those in formula. Samples were chosen among both hydrolyzed and non-hydrolyzed infant formulas as follows: Enfapro step 2, Similac Advance step 1, Similac Go & Grow- Step 2, Enfamil step 1, Parent’s Choice Gentle 0-12 months, Isomil soy protein step 2, Isomil soy protein step 1, Enfamil A+ step 1, Nestle Good Start 2 (partially hydrolyzed), Nestle Good Start 0+ (partially hydrolyzed), Alimentum (extensively hydrolyzed), Nutramigen A+ (extensively hydrolyzed). Each formula was sampled six times and each sample was obtained from a different container of formula that was randomly acquired from different stores in the greater Toronto area.

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada and the Natural Health Product Directorate of Health Canada. Written informed consents were obtained from all subjects after a thorough explanation of the study details and each subject was free to withdraw anytime during the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki (1997).

2.3.1. Laboratory Analyses

All laboratory analyses were conducted at TransBioTech by technicians blinded to study condition. Methods for each analysis are described below:
Free tyrosine in plasma and breast milk samples

Free tyrosine analysis was performed by high pressure liquid chromatography with fluorescence detection (HPLC-FLD). Sample proteins were precipitated over 30 minutes on ice after mixing each sample with two parts of cold methanol. Samples were then centrifugated and the supernatant was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane prior to analysis. A Phenomenex Gemini C6-Phenyl column, 4.6 x 250 mm, 3 µm, heated at 40°C, was used for the separation in gradient mode at flow rate of 1 mL/minute. A linear gradient separation was performed with (A) a 20 mM pH=5.4 sodium acetate buffer and (B) acetonitrile, beginning at 2% B for 2 minutes, followed by a gradient from 2% B to 100% B in 8 minutes. The fluorescence detector was set at 274 nm for the excitation and at 320 nm for the emission.

Total tyrosine in breast milk

For total tyrosine analysis, 1 mL of concentrated hydrochloric acid containing 2 mg/mL of phenol was added to 1 mL of sample in a vacuum hydrolysis tube. The vacuum was applied for 10 minutes and hydrolysis was performed at 110°C for 23 hours. The tubes were cooled on ice and the acid was neutralized using NaOH. The solution was filtered and the volume was brought to 50 mL by adding a 1M pH = 5.4 sodium acetate buffer prior to analysis. Total tyrosine was assayed with the HPLC-FLD procedure described above.
Free tyrosine in infant formulas

For the analysis of free tyrosine in infant formulas, proteins were precipitated for 30 minutes on ice after mixing one part of sample with two parts of methanol. After centrifugation at 4000 rpm for 10 minutes, the supernatant was filtered through a 0.45 µm PTFE membrane and placed in a HPLC vial. O-phthaldialdehyde (OPA) was used to selectively derivatize the amino acids from the matrix. The OPA solution was prepared fresh each day as follows. To 0.5 mL of methanol was added 65 mg of OPA and, after dissolution, 2.8 mL of a 0.6 M pH = 10.2 tetraborate buffer and 65 µL of 2-mercaptoethanol. This solution was mixed with an equal volume of a Brij solution (1 mL of 30% Brij 35 diluted with 14 mL of tetraborate buffer pH = 10.2) to obtain the OPA derivatization solution. The HPLC injector was programmed to successively mix 2.5 µL of tetraborate buffer pH = 10.2 with 0.5 µL of OPA solution, 0.5 µL of sample and, after 2 minutes, 18 µL of a 0.3 M tetraborate buffer pH = 2.7. After mixing, this solution was injected on an Alltech Adsorbosphere OPA-HS column, 4.6 x 100 mm, 5 µm with a flow rate of 1.0 mL/min. The mobile phase was a mixture of (A) a 20 mM pH = 5.4 sodium acetate buffer, (B) methanol and (C) tetrahydrofuran (THF). The gradient began with 5% B and 5% C, evolving in 20 minutes to 65% B and 2% C. For detection, the excitation and emission wavelengths were set at 325 nm and 465 nm, respectively.
2.3.2. **Statistical analyses**

The primary analysis was a repeated measures analysis of variance for total levels of tyrosine in breast milk over time. Group (defined as no supplement, or each dose of tyrosine) was entered as a between subject factor in the analysis. Mean and standard deviation for total tyrosine in breast milk was also calculated for each time point in all dosing groups. Similarly, repeated measures analysis of variance was also applied for free tyrosine levels in breast milk over time. Group (as defined previously) was also entered as a between subject factor in the analysis. For tyrosine levels in plasma over time, also repeated measures analysis of variance was used with group being entered as a between subject factor in the analysis.

The free tyrosine levels in formula were plotted as bar graphs. Analysis of variance (ANOVA) was used to compare each formula with the study groups. Post-hoc Fisher's least significant difference (LSD) was also applied in order to look at each specific group comparison among the formula and breast milk samples.
2.4. Results

Twenty four healthy breastfeeding women were recruited into 4 groups (2, 5, and 10 grams of tyrosine and a group with no supplements) (Figure 2-1). The mean age of participants was 30.5±4.7. Tyrosine supplements were well tolerated and no adverse events were observed in any of the dosing groups. At baseline, the mean total tyrosine level in breast milk, mean tyrosine level in plasma, and mean free tyrosine level in breast milk were 24.26±5.54 mg/dl, 11.16±3.07 microgram/ml and 0.16±0.08 mg/dl respectively. One-way multivariate analysis of variance revealed that baseline values of free and total tyrosine in breast milk and plasma free tyrosine were not different among the groups.
Figure 2-1. Subject Recruitment Detail

- Inquired About the Study = 56
  - Unable to Contact = 9
  - Phone Screened = 47
    - Not Eligible = 5
      - Not Interested = 12
    - Provided Consent = 30
      - Dropout = 6
  - Total Subjects Randomized = 24
    - Control = 6
    - 2 grams Tyrosine = 6
    - 5 grams Tyrosine = 6
    - 10 grams Tyrosine = 6
2.4.1. Total Tyrosine in Breast Milk

Repeated measures of ANOVA revealed no effect of time by group interaction ($F(9,60)=1.87, p=0.074$); there was a trend observed towards a slight reduction in total tyrosine, especially in the two groups who had received higher doses (**Figure 2-2**). In addition, repeated measures of ANOVA showed no effect of group across the cumulative samples taken ($F(3,20)=1.24, p=0.321$).
Figure 2-2. No Rise in Total Tyrosine Levels in Breast Milk After Oral Tyrosine Supplements.

Repeated measures analysis of variance found no effect of group on repeated measurement of total tyrosine levels ($F (3,20)=1.24, p=0.321$). Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
2.4.2. Maternal Plasma Tyrosine

Repeated measures of ANOVA showed a significant effect of group (F (3,20)=33.67, \( p<0.005 \)). The post hoc LSD test revealed that the 10g tyrosine group had a significantly higher plasma tyrosine compared to the other groups (\( p<0.001 \)), the 5g tyrosine group had significantly higher plasma tyrosine compared to the non-supplement group and significantly lower values compared to the 10g tyrosine group (\( p=0.002, \ p<0.005 \) respectively), 2g tyrosine group had significantly higher values compared to non-supplement group and significantly lower values compared to the 10g tyrosine group (\( p=0.025, \ p<0.005 \) respectively). Repeated measures of ANOVA also showed a significant time by group interaction in plasma tyrosine (F (18,120)=6.37, \( p<0.005 \)) (Figure 2-3).

Plasma tyrosine was maximal around 4 hours for 10g tyrosine whereas for 5g tyrosine the peak plasma tyrosine was between 4-6 hours. For 2g tyrosine the peak was around 2-3 hours.
Figure 2-3. Elevation in Plasma Tyrosine Levels Over Time After Oral Tyrosine Supplements.

Repeated measures analysis of variance showed a strong effect of group on plasma levels of free tyrosine (F (3,20)=33.67, p<0.005). Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
2.4.3. Free Tyrosine in Breast Milk

Repeated measures of ANOVA showed a significant effect of group (F (3,20)=10.25 \( p<0.005 \)). The post hoc LSD test revealed that the 10g tyrosine group had a significantly higher free tyrosine in breast milk compared to the other groups (\( p<0.001 \)). Repeated measures of ANOVA also showed a significant time by group in free tyrosine in breast milk (F (9,60)=5.26, \( p<0.005 \)) (Figure 2-4).

Peak concentration of free tyrosine in breast milk after 10g dose occurred at 4 hours, whereas for 2g and 5g tyrosine maximum free tyrosine levels in breast milk occurred at 6 hours.
Figure 2-4. Free Tyrosine Levels in Breast Milk are Elevated After Oral Tyrosine Supplements.

Repeated measures analysis of variance showed a strong effect of group on breast milk levels of free tyrosine ($F(3,20)=10.25\ p<0.005$). Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
2.4.4. Free Tyrosine in Infant Formula

Twelve popular marketed infant formulas, chosen from hydrolyzed and non-hydrolyzed formulas, were measured for their free tyrosine contents. Six different samples were tested for each formula brand. Two brands had free tyrosine levels below the limit of detection (<0.01mg/100ml) and for the other 10, free tyrosine ranged from 0.18 to 20.51mg/100ml across the samples. Free tyrosine concentration in our highest supplemented group was significantly lower than the 2 extensively hydrolyzed infant formulas tested ($p<0.005$), and was similar to free tyrosine measured in the partially hydrolyzed formulas Good Start 0+ and Good Start 2 ($p=0.790$, $p=0.739$ respectively). Free tyrosine in 6 non-hydrolyzed formulas was significantly lower than free tyrosine in breast milk of subjects on 10g tyrosine ($p<0.05$) (Figure 2-5).
Figure 2-5. Free Tyrosine in Common Infant Formulas and in Breast Milk After Oral Tyrosine Supplementation.

Mean and standard deviation are presented. Free tyrosine in breast milk even with the highest dose of 10g oral tyrosine was significantly lower than free tyrosine in the 2 extensively hydrolyzed infant formulas tested (Alimentum and Nutramigen A+) (Analysis of variance, post-hoc comparisons, Fisher's least significant difference (LSD), $p<0.005$) and similar to partially hydrolyzed formulas tested (Good Start 0+ and Good Start 2).
2.5. Discussion and Conclusion

To our knowledge, this is the first study to investigate the effect of oral tyrosine supplements on total and free tyrosine contents of breast milk in breastfeeding mothers. The primary findings of this study are that tyrosine supplements tested did not increase total tyrosine in breast milk, and that free tyrosine (2% of total tyrosine) increased with maternal tyrosine supplementation but the free tyrosine elevation was similar to the range found in generally available infant formulas.

The lack of effect of oral tyrosine upon total tyrosine in breast milk was in accordance with the proposed hypotheses, a finding that has never been previously demonstrated. The average amount of total tyrosine in breast milk previously reported had a range from 24 to 63 mg/100ml (Yamawaki et al. 2005). In our study the average total tyrosine at baseline was 24.2 mg/100 ml and the highest amount measured after supplementation was 38 mg/100 ml, (in the range reported in women without supplements). The total nitrogen (crude protein) in milk consists of non-protein nitrogen fraction and protein nitrogen fraction. Non-protein nitrogen accounts for 20-25% of the total nitrogen content (Emmett and Rogers 1997) and is mostly derived from maternal plasma. Free amino acids are part of the non-protein nitrogen fraction, which also includes other compounds such as peptides, uric acid and creatine. Free amino acids represent around 4% of total amino acids in breast milk and among them tyrosine is often present in relatively lower concentrations. Previous data show that the protein content in human milk is relatively constant as compared to the non-protein nitrogen fraction, which is often affected by maternal variables. Studies looking at the effect of
fasting or diets with different protein contents have mostly reported no change in total protein of milk, despite the changes that might been seen in free levels (Bener et al. 2001; Lonnerdal 1986; Rakicioglu et al. 2006). Free tyrosine incorporates less than 2% of total tyrosine in breast milk. Therefore, the reason that total tyrosine in breast milk is not increased as opposed to free tyrosine is likely because of the characteristic linkage of amino acids in chains to form a protein structure.

The effect of oral tyrosine supplementation on free tyrosine in breast milk has not been previously evaluated. Free tyrosine in breast milk has previously been reported between 0 to 3.58 mg/100 ml with the variation likely mainly related to diet (Agostoni et al. 2000a; Carratu et al. 2003). In our study, free tyrosine ranged from 0.03 to 2.81 mg/100 ml, which would suggest that despite the elevation in free tyrosine due to tyrosine supplementation, the levels are within the range reported without supplementation.

The effect of oral tyrosine upon plasma tyrosine had not been previously examined in breastfeeding mothers. However, studies in other human populations have reported a peak plasma tyrosine level between 2 to 3 hours after supplement ingestion (Melamed et al. 1980; Shurtleff et al. 1994; Tumilty et al.). Our study indicated that maternal plasma tyrosine significantly increased with the magnitude of the tyrosine supplements. This effect was maximal between 3 to 5 hours (depending on the dose), with a modest delay compared to previous studies. These data also showed a similar trend, with an elevation both in the breast milk free tyrosine and plasma tyrosine with each dose, supporting a relationship between free tyrosine in breast milk and plasma tyrosine directly which was also observed in our sample.

This is also the first study that measured free tyrosine in multiple, random, samples of marketed infant formulas in North America. While there has been a recent report of free
amino acid levels in some infant formulas in North America, it only looked at a single random sample in the 8 different formula brands (Ventura et al. 2012). Investigators from Italy (12 different formulas, 2 samples per each formula) and Spain (18 different formulas, 1 sample per each formula), measured amino acids in infant formulas marketed in Europe (Agostoni et al. 2000b; Alegria et al. 1999). Although the number of samples in the earlier studies is small and comparisons are difficult, the findings are not contradictory to ours. Comparison of free tyrosine in breast milk in our subject groups with some marketed infant formulas showed that free tyrosine in breast milk is in the range of free tyrosine in infant formula (between 0.18-20.51 mg/100 ml). Infant formulas come in different types: cow's milk, soy protein based, partially hydrolyzed, extensively hydrolyzed and amino acid based formulas. It would be expected that hydrolyzed formulas would have higher free amino acids compare to other formulas and to our study groups. Hydrolyzed formulas are considered as hypoallergenic formulas as they have larger protein chains broken down for better digestion, and free amino acid-based formulas do not include protein chains at all. Partially hydrolyzed formulas differ from extensively hydrolyzed formulas in that their protein chains can be longer. These groups of approved formulas by regulatory agencies are the ones that had much higher free tyrosine than the breast milk from our supplemented groups. Free tyrosine results found in the breast milk samples of our study are closer to non-hydrolyzed and partially hydrolyzed formulas and much lower than the extensively hydrolyzed formulas.

A limitation of this study is that the duration for sampling may have been insufficient for tyrosine levels to return to baseline for all assays. However, it was noted that the plasma levels in mothers were near the baseline levels at the end of the protocol, and that the total
tyrosine levels in breast milk did not exhibit any change. This duration was chosen as the mothers were most comfortable with bottle-feeding their infants for this length of stay, and with the highest dose of 10g oral tyrosine in our study we were able to detect the peak and subsequent decrease of tyrosine levels in plasma and free tyrosine levels in breast milk.

In conclusion, we think that there is good rationale to further investigate 10g of oral tyrosine as part of a dietary supplementation strategy to prevent PPD. This perspective is based on several factors: the first is that this dose did not increase total tyrosine in breast milk; second is that previous human studies demonstrate that 100-150 mg/kg/day reduces adverse mood and performance impairments due to environmental stresses (Banderet and Lieberman 1989; Deijen and Orlebeke 1994; Glaeser et al. 1979; Melamed et al. 1980; Shurtleff et al. 1994; Stone 1975; Yeghiayan et al. 2001); and finally oral tyrosine raises plasma tyrosine in women, which can eventually increase the concentration of tyrosine in the brain and increase the synthesis of the central catecholamines dopamine and norepinephrine (Stone 1975). Hence, this supplementation may be applicable as a strategy to overcome the high MAO-A levels in early postpartum. Although an effect was seen on free tyrosine, it accounts for only 1-2% of total tyrosine in breast milk, and even the highest level of free tyrosine in breast milk measured in our subjects was comparable to several non-hydrolyzed infant formulas and less than 1/10 of the levels in extensively hydrolyzed infant formulas. Given the high prevalence rate of PPD, its significant long-term morbidity, and lack of successful widespread prevention strategies, as well as the previously demonstrated beneficial effects of oral tyrosine upon mood and cognitive performance, the present study supports further investigation of oral tyrosine as a prevention strategy for PPD, based upon its lack of effect on its total levels in breast milk.
Chapter 3  No Effect of Oral Tryptophan and Oral Alpha-lactalbumin on Total Tryptophan Levels of Breast Milk

This work has been accepted with revisions by the European Journal of Neuropsychopharmacology as of February 17, 2015


* With the addition of recruitment detail
3.1. Abstract

**Purpose:** Postpartum depression (PPD) is the most common complication of childbearing with a 13% prevalence rate. Sleep disturbances are also common, particularly during early postpartum. In theory, tryptophan could improve sleep and reduce depressed mood in early postpartum; however, the first step in clinical development of tryptophan for use in postpartum is to measure the effect of oral tryptophan on its concentrations in breast milk, which is presently unknown.

The aims were to investigate the effect of oral tryptophan and alpha-lactalbumin, a protein with high tryptophan concentration, on total and free tryptophan levels in breast milk and plasma, and to compare free tryptophan levels in breast milk with those in common infant formulas.

**Methods:** Thirty healthy breastfeeding women were randomly allocated to receive 2g or 4g of tryptophan, or, 20g or 40g of alpha-lactalbumin or no supplement. Free tryptophan levels were also measured in 12 different infant formulas.

**Results:** Total tryptophan in breast milk was unaffected by oral administration of tryptophan or alpha-lactalbumin (repeated measures of ANOVA (rANOVA), group effect, $p=0.232$). Both tryptophan and alpha-lactalbumin were associated with greater free tryptophan levels in breast milk (rANOVA, group effect: $p<0.005$) (representing 2% of total tryptophan) but these concentrations were within the range of commonly used infant formulas.

**Conclusion:** In contrast to most sleep inducing medications, tryptophan does not affect its total concentration in breast milk. These results support further investigation of dietary
tryptophan and alpha-lactalbumin as part of a dietary supplementation approach to address sleep disturbances in postpartum and reduce risk of PPD.

3.2. Introduction

Two important problems of childbearing are clinical level postpartum depression (PPD) and sleep disturbances with the former having a 13% prevalence rate and the latter having a 30% prevalence rate (Nishihara et al. 2001; O'Hara and Swain 1996). In addition depressed mood and disrupted sleep are often comorbid in the postpartum period (Ohayon and Roth 2003; Roth 2007). While sleep disturbance is a symptom of PPD, it can also lead to lower mood, fatigue, anxiety and reduced ability to cope with stress, further increasing the risk of PPD (Dorheim et al. 2009; Ross et al. 2005). In addition, chronic sleep deprivation and fragmentation, interferes with normal cognitive function, and is associated with poorer health outcomes (Dorheim et al. 2009; Durmer and Dinges 2005).

There are currently no standard prevention strategies for PPD in healthy women (Goodman 2004). General psychosocial support during postpartum is associated with a reduced level of symptoms but not the prevalence of diagnostically established PPD. In regards to dietary supplements, there have been some preliminary investigations of the benefit of fish oil, which, to date, have not yielded positive results, although they may be underpowered (Amminger et al. 2010; Levant 2011).

Given the multiple biological underpinnings implicated in major depressive disorder, it is possible that combinations of dietary supplements with different targets may have a role in
preventing PPD. For such a strategy the first step is to determine whether each individual ingredient of such a supplement has an effect upon breast milk. In the present study, we assess this issue for tryptophan, and a protein with very high tryptophan concentration, \alpha-lactalbumin. The basis for oral tryptophan is that it will increase the ratio of tryptophan to other large neutral amino acids (LNAA) in blood plasma thereby increasing its transport across the blood brain barrier, leading to greater precursor availability and greater serotonin synthesis. There is a link between plasma tryptophan and mood such that oral supplements rich in the amino acid tryptophan or protein containing high concentrations of tryptophan may be associated with happier mood in healthy people (Fernstrom 2012; Markus et al. 2008) and induced depletion of tryptophan can create a sad mood, particularly in those vulnerable to major depressive episodes (Delgado et al. 1994; Young et al. 1985). Another reason oral tryptophan has therapeutic potential in early postpartum is that the primary removal process of serotonin is enhanced at this time: during early postpartum, levels of monoamine oxidase A (MAO-A), the enzyme mainly responsible for serotonin metabolism are elevated by more than 40% in regions such as the prefrontal and anterior cingulate cortex, which are known to regulate affect (Sacher et al. 2010).

Most randomized, double blind, placebo controlled studies of oral tryptophan to treat insomnia, particularly at doses of at least 1g day report a positive finding of decreased sleep latency (for review see (Fernstrom 2012; Silber and Schmitt 2010)), and thus, has the potential to reduce sleep disturbances during postpartum. Although there are a number of non-pharmacological interventions for sleep disturbances in postpartum including help with infant care, cognitive behavioural therapy (CBT), and education regarding sleep hygiene, among the interventions systematically studied success rates are often lower than 50% (Lee
and Gay 2011; Stremler et al. 2013; Swanson et al. 2012). Pharmacological options such as benzodiazepines, zopiclone, trazodone, mirtazapine and tryptophan have been considered, however, all of these, with the exception of tryptophan have been shown to cross into breast milk (Briggs et al. 2011). To the best of our knowledge the effect of tryptophan on its levels in breast milk is unknown.

While there are reasons to investigate tryptophan to reduce insomnia in postpartum, or to prevent PPD as part of a dietary supplement, the first logical step is to assess its effects upon its concentrations in breast milk.

While in some countries such as United States tryptophan is considered as a natural health product, in some countries such as Canada, tryptophan doses above 220 mg are considered a drug. Therefore, there is also need to consider regulator practices across countries and thus potential substitutions were considered. Alpha-lactalbumin is a whey derived protein rich in the amino acid tryptophan and therefore might have the potential to be used as a source of tryptophan (Heine et al. 1996; Markus et al. 2000). Studies have shown that alpha-lactalbumin supplementation can increase the plasma ratio of tryptophan to large neutral amino acids (LNAA) between 50% to 130% (Booij et al. 2006; Markus et al. 2002; Markus et al. 2000; Scrutton et al. 2007). This elevation may result in an increase in tryptophan transportation across the blood-brain-barrier; thus enhancing brain serotonin synthesis function (Beulens et al. 2004; Booij et al. 2006; Lehnert and Wurtman 1993; Markus et al. 2005; Markus et al. 2000; Merens et al. 2005; Scrutton et al. 2007). In addition, alpha-lactalbumin was reported to improve mood during a stressful task in stress-prone individuals (Markus et al. 2002; Markus et al. 2000).
The main objective of this study was to investigate the effect of a single dose of oral tryptophan or alpha-lactalbumin on total tryptophan levels in breast milk, at doses known to raise tryptophan in blood plasma. The second objective was to investigate the effects of oral tryptophan and alpha-lactalbumin upon free tryptophan levels in breast milk. In addition, to assess how the change in free tryptophan compares to common exposures in infants, the third objective was to systematically measure free tryptophan levels across different approved infant formulas frequently given in North America, as a comparator.

3.3. Methods

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada and the Natural Health Product Directorate of Health Canada. Written informed consent was obtained from all subjects after a thorough explanation of the study. Each subject was free to withdraw anytime during the study (1997).

Healthy breastfeeding women aged 18 to 40 were recruited through advertisements in the Greater Toronto Area, Canada. Subjects were eligible if they were healthy, currently breastfeeding during 2 to 24 months postpartum, medication-free and not taking any over the counter medication. They were excluded if they were currently pregnant (screened with urine pregnancy test), used any investigational medicinal product or herbal medication within the previous 8 weeks, gave a self report of psychiatric or current medical illnesses, suicide attempts, substance abuse (tested with urine drug screen), had known hypersensitivity to
components in the proposed treatment, had a history of severe drug allergy or drug hypersensitivity involving an anaphylactic reaction.

Subjects were randomly assigned to one of five groups (6 subjects per group): a single dose of either 2g of tryptophan, 4g of tryptophan, 20g of alpha-lactalbumin (containing almost 1g of tryptophan), 40g of alpha-lactalbumin (containing almost 2g of tryptophan) or be in the control group without receiving any supplements.

The tryptophan capsules were obtained from Apotex and each capsule contained 1g of tryptophan (Apo-tryptophan by Apotex Incorporated, Toronto, Ontario, Canada, DIN# 02248539). Apo-tryptophan has been approved for use in Canada by Health Canada based on purity, consistency of content and lack of toxins. The investigator brochure for this product may be found at Health Canada’s website (www.hc-sc.gc.ca / January 25, 2015). Tryptophan capsules were taken with water. Alpha-lactalbumin powder was obtained from Davisco Foods International Inc. (Eden Prairie, MN, USA). Davisco alpha-lactalbumin has 4.8 grams of tryptophan per 100 grams of protein and, to the best of our knowledge, is the purest, isolated form of bovine alpha-lactalbumin commercially available. Of the total protein content, at least 90% is alpha-lactalbumin (www.daviscofoods.com). Oral administration of Alpha-lactalbumin by Davisco consistently demonstrates its ability to increase the ratio of plasma tryptophan to other large neutral amino acids (TRP/LNAA) (Markus et al. 2008; Scrutton et al. 2007). This product also met Health Canada standards for purity, quality and lack of toxin (product standards provided by manufacturer).

In order to improve taste, alpha-lactalbumin powders were mixed with 1 container of 111g Mott’s Fruitsations berry puree (Canada Dry Mott’s Inc., Mississauga, Ontario, Canada). It contains 13 grams of carbohydrate, which is only 4% of total daily value.
Side effects of tryptophan are rare, but more common at higher doses. A common side effect from l-tryptophan use is drowsiness. Other less common tryptophan side effects include nausea, dizziness, and dry mouth.

Subjects came for 2 visits: the first was a screening visit. For the second visit, subjects attended the laboratory based on the schedule in Table 3-1. Dietary intake was standardized for all subjects to enhance the ability to detect the effect of different doses of tryptophan and alpha-lactalbumin supplements. Subjects fasted overnight from 11 pm the night before and were only allowed to have water before coming to the lab. During their second visit, all subjects were provided with a standardized low protein breakfast and lunch. Four breast milk samples (1 prior to supplement intake and 3 over the next 6 hours) and 7 blood samples (2 prior to supplement intake and 5 over the next 6 hours) were collected (Table 3-1). At each time point, 30ml of breast milk were obtained with an electric pump (Medela Symphony Breast pump), transferred into sterile polypropylene vials, coded and frozen at -80°C until assayed. Also 10ml of maternal blood at each time point were sampled: a onetime venipuncture and saline lock was completed first. Then sampling was conducted via the saline lock. During the second visit, mothers did not breastfed their infant and infants were bottle fed until after the last sample was taken.
Concentration of free tryptophan was also measured in 12 popular marketed infant formulas in order to compare free tryptophan levels in breast milk with those in formula. Each formula was sampled 6 times and each sample was obtained from a different container of formula that was randomly acquired from different stores in the greater Toronto area. Samples were chosen among both hydrolyzed and non-hydrolyzed infant formulas as follows: Enfapro step 2, Similac Advance step 1, Similac Go & Grow- Step 2, Enfamil step 1, Parent’s Choice Gentle 0-12 months, Isomil soy protein step 2, Isomil soy protein step 1, Enfamil A+ step 1, Nestle Good Start 2 (partially hydrolyzed), Nestle Good Start 0+ (partially hydrolyzed), Alimentum (extensively hydrolyzed), Nutramigen A+ (extensively hydrolyzed).

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
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<tbody>
<tr>
<td>8:00 am</td>
<td>Subject Arrival</td>
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<tr>
<td>8:15 am</td>
<td>Plasma Sampling</td>
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<tr>
<td>8:30 am</td>
<td>Plasma Sampling / Breast Milk Sampling</td>
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<tr>
<td>8:40 am</td>
<td>Tryptophan or Alpha-lactalbumin Intake</td>
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<tr>
<td>9:00 am</td>
<td>Breakfast (low-protein)</td>
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<td>9:30 am</td>
<td>Plasma Sampling</td>
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<td>10:30 am</td>
<td>Plasma Sampling / Breast Milk Sampling</td>
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<tr>
<td>11:30 am</td>
<td>Plasma Sampling</td>
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<tr>
<td>12:00 pm</td>
<td>Lunch (low-protein)</td>
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<td>12:30 pm</td>
<td>Plasma Sampling / Breast Milk Sampling</td>
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<tr>
<td>2:30 pm</td>
<td>Plasma Sampling / Breast Milk Sampling</td>
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3.3.1. Laboratory Analyses

Free tryptophan in plasma and breast milk samples

Free tryptophan was separated and quantified by high pressure liquid chromatography with fluorescence detection (HPLC-FLD) adapted from the Iizuka et al., Carratu et al. and Delgado-Andrade et al. methods (Carratu et al. 2003; Delgado-Andrade et al. 2006; Iizuka et al. 2011). Unlike the analysis of other amino acids, derivatization with a fluorophore prior to the analysis was not needed, since those two compounds exhibit native fluorescence. For samples, proteins were precipitated over 30 minutes on ice after mixing the sample with two parts of methanol. Samples were then centrifuged at 4000 rpm for ten minutes, the supernatant was filtered on a 0.45 µm polytetrafluoroethylene (PTFE) membrane and 10 µL of the sample was injected directly in the HPLC-FLD system. A Phenomenex Gemini C6-Phenyl column, 4.6 x 250mm, 3µm was used for the separation in gradient mode at flow rate of 1 mL/minute. The column was heated at 40°C during the analysis. Mobile phase A was a 20mM sodium acetate buffer at pH=5.4 and mobile phase B was acetonitrile. The initial condition was 2% B for 2 minutes followed by a gradient from 2% B to 100% B in 8 minutes. The column was kept for 2 minutes at 100% B, brought back to 2% B in one minute and re-equilibrated at this composition for 4 minutes. The fluorescence detector was set at 274 nm for the excitation. For the emission, the detector was set at 340 nm during the 5 first minutes for the detection of the tryptophan.
**Total tryptophan in breast milk**

An aliquot of 100 µL of a 1000 mg/mL internal standard solution was added to 300 µL of milk samples in a nalgene tube sealed with a PTFE septum cap. The basic hydrolysis was conducted according to Delgado-Andrade et al. Procedure (Delgado-Andrade et al. 2006). Briefly, 4 mL of NaOH 4N and 200µL of 5-methyl-tryptophane (1250 mg/L) as internal standard was added to the tube. The tube was kept on ice for at least 10 minutes and vacuum was then applied using a needle piercing through the septum. The tube was heated at 110°C for 18 hours on a dry bath. The solution was then cooled on ice and neutralized with 5 mL of HCl 3N. The solution was filtered on Whatman #4 paper and the volume was brought to 50 mL by adding a 1M sodium acetate buffer at pH=5.4. Prior to the analysis the solution was filtered on 0.45µm PTFE filter. For the separation, a Phenomenex Kinetex C18 4.6 x 250mm, 2.6µm was used. The flow rate was set to 0.8 mL/minute and the temperature at 40°C. The analysis was performed with the same mobile phases as in the previous sections but the gradient was modified. Initial conditions were 2% B for 0.5 minute, followed by a linear gradient from 2% to 7% B in 4.5 minutes, and a second gradient from 7% to 13% B over 10 minutes. The column was washed during 2 minutes at 100% B, brought back to 2% B in one minute and re-equilibrated during 4 minutes at this composition. The same detection parameters were used but the emission wavelength was switched from 320 nm to 340 nm at 4 minutes.
Free tryptophan in infant formulas

Because of the complexity of the infant formula matrix, the approach described previously could not be employed. The procedure from Agilent Technologies was used for the quantitation of tryptophan (Henderson et al. 2000). For the analysis of free tryptophan, the proteins were first precipitated for 30 minutes on ice after mixing one part of sample with two parts of methanol. After centrifugation at 4000 rpm for 10 minutes, the supernatant was filtered on a 0.45 µm PTFE membrane and placed in a HPLC vial. Then, a derivatization methodology with o-phtaldehyde (OPA) was used to selectively detect the amino acids from the matrix. The OPA derivatization solution was prepared fresh each day as follows. To 0.5 mL of methanol was added 65 mg of OPA and, after dissolution, 2.8 mL of a 0.6 M tetraborate buffer at pH=10.2 and 65 µL of 2-mercaptoethanol. This solution was mixed to an equal volume of a Brij 35 solution (1 mL of 30% Brij 35 diluted with 14 mL of the tetraborate buffer pH=10.2) to obtain the OPA derivatization solution. The HPLC injector was programmed to successively mix 2.5 µL of tetraborate buffer at pH=10.2 with 0.5 µL of OPA derivatization solution, 0.5 µL of sample and, after two minutes, 18 µL of a 0.3 M tetraborate buffer at pH=2.7. After mixing, this solution was injected and analyzed on an Alltech Adsorbosphere OPA-HS column, 4.6 x 100 mm, 5 µm with a flow rate of 1.0 mL/min. The mobile phase was a mixture of (A) a 20 mM sodium acetate buffer at pH=5.4, (B) methanol and (C) tetrahydrofuran (THF). The gradient began with a composition of 90% A, 5% B and 5% C, evolving in 20 minutes to 33% A, 65% B and 2% C. The composition was brought back in one minute to the initial conditions and re-equilibrated for 5 minutes prior to the next analysis. For detection, the excitation and emission wavelengths were set at 325 nm and 465 nm, respectively.
3.3.2. Statistic Analyses

To determine whether any of the oral supplements had a differential effect upon total concentrations of tryptophan in breast milk, repeated measures analysis of variance (rANOVA) with total tryptophan level as the dependent variable and group as the predictor variable was applied. Also, to assess whether overall total tryptophan levels differed among groups the area under the curve (AUC) for total tryptophan was found for each subject and then AUC was applied as a dependent variable in an analysis of variance (ANOVA) with group as the predictor variable. A similar approach was applied to assess the effect of the oral supplements on free tryptophan levels in breast milk and free tryptophan levels in blood plasma.

Analysis of variance was used to compare free tryptophan levels in each formula and each sample set of breast milk taken after supplementation. Post-hoc Fisher's least significant difference (LSD) was also applied for specific group comparisons among the formula and breast milk samples.
3.4. Results

Thirty healthy breastfeeding mothers completed the study protocol (Figure 3-1). Tryptophan at 2g and all alpha-lactalbumin doses were well tolerated and no adverse effects were observed in any of the dosing groups. Three out of 6 subjects receiving 4g tryptophan felt nauseous between 30 minutes to 50 minutes after ingesting the oral dose. The Post hoc LSD test revealed a significant difference between the group receiving 4g tryptophan and other groups in regards to side effects due to nausea ($p<0.005$).

At baseline, the mean total tryptophan level in breast milk, mean free tryptophan level in plasma, and mean baseline free tryptophan in breast milk were $23.33 \pm 3.34$ mg/dl, $11.6 \pm 2.58$ microgram/ml and $0.04 \pm 0.21$ mg/dl respectively. This was consistent with what has been previously reported in literature (Chavalittamrong et al. 1981; Scott et al. 1990; Wurtman and Fernstrom 1979).
Figure 3-1. Subject Recruitment Detail

- Inquired About the Study = 59
- Unable to Contact = 8
- Phone Screened = 51
- Not Eligible = 6
- Not Interested = 14
- Provided Consent = 31
- Dropout = 1
- Total Subjects Randomized = 30
  - Control = 6
  - 2 grams Tryptophan = 6
  - 4 grams Tryptophan = 6
  - 20 grams Alphalactalbumin = 6
  - 20 grams Alphalactalbumin = 6
3.4.1. Total Tryptophan Levels in Breast Milk

There was no main effect of group (F (4,25)=0.22, p=0.927) or time (with a trend towards reduction) (3,75)=2.71, p=0.051) on total tryptophan concentrations in breast milk, and the time by group interaction was not significant (F (12,75)=1.31, p=0.232) (Figure 3-2).

There was no effect of group on the AUC of total tryptophan in breast milk (F (4,25)=0.21, p=0.928).
Figure 3-2. Total Tryptophan Concentrations in Breast Milk after Oral Tryptophan or Alpha-lactalbumin (a-lac) Supplements.

There was no effect of group on repeated measurement of total tryptophan levels in breast milk ($F(12,75)=1.31$, $p=0.232$). Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
3.4.2. Free Tryptophan Levels in Plasma

There was a significant effect of group (F (4,25)=34.68, p<0.001) and time (F (6,150)=94.7, p<0.001) on free tryptophan levels in plasma. The time by group interaction was also significant (F (24,150)=15.62, p<0.001) (Figure 3-3). Repeated measures of ANOVA showed a significant effect of group (F (4,25)=34.68, p<0.005). The Post hoc LSD test revealed that the 4g tryptophan group had a significantly higher plasma tryptophan compared to all other groups (p<0.005), but not compared to 2g tryptophan (p=0.154). The group receiving 2g tryptophan had significantly higher plasma tryptophan compared to no supplement and 20g and 40g alpha-lactalbumin (p<0.005), but was not significantly lower from 4g tryptophan (p=0.154). The group receiving 40g alpha-lactalbumin had significantly lower plasma tryptophan compared to 2g and 4g tryptophan groups (p<0.005) and significantly higher plasma tryptophan compared to 20g alpha-lactalbumin and no-supplement group (p=0.007 and p<0.005 respectively). The group receiving 20g alpha-lactalbumin had a significantly lower plasma tryptophan compared to the 2g and 4g tryptophan groups (p<0.005) and 40g alpha-lactalbumin group (p=0.007) and significantly higher plasma tryptophan compared to no supplement group (p<0.005).

There was a significant effect of group on the AUC of free tryptophan in plasma (F (4,25)=36.25, p<0.001). The Post hoc LSD test revealed that the 4g l-tryptophan group had a significantly higher AUC of plasma tryptophan compared to all other groups (p<0.001), except the 2g l-tryptophan (p=0.15). The group receiving 2g l-tryptophan had significantly higher AUC of plasma tryptophan compare to no supplement (p<0.001), and 20g and 40g alpha-lactalbumin (p<0.005), but was not significantly lower from 4g l-tryptophan (p=0.15).
The group receiving 40g alpha-lactalbumin had significantly lower AUC of plasma tryptophan compared to 2g and 4g l-tryptophan groups \((p<0.005)\) and significantly higher compared to 20g alpha-lactalbumin and no-supplement group \((p=0.007\) and \(p<0.001\) respectively). The group receiving 20g alpha-lactalbumin had a significantly lower AUC of plasma tryptophan compared to the 2g and 4g l-tryptophan groups \((p<0.001)\) and 40g alpha-lactalbumin group \((p=0.007)\) and significantly higher compared to no supplement group \((p<0.05)\).

Plasma tryptophan was maximal around 1-2 hours for 2g and 4g tryptophan and then decreased to near baseline values after 6 hours. The peak for plasma tryptophan with alpha-lactalbumin doses was between 2-2.5 hours and then decreased to near baseline values after 6 hours.
Figure 3-3. Plasma Free Tryptophan Concentrations after Oral Tryptophan or Alpha-lactalbumin (a-lac) Supplements.

There was a significant effect of group on plasma levels of free tryptophan ($F(24,150)=15.62$, $p<0.001$). Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
3.4.3. Free Tryptophan Levels in Breast Milk

There was a significant effect of group (F (4,25)=20.43, p<0.001) and time (F (3,75)=43.11, p<0.001) on free tryptophan levels in breast milk. The time by group interaction was also significant (F (12,75)=13.64, p<0.001) (Figure 3-4). The Post hoc LSD test revealed that the 4g tryptophan group had a significantly higher free tryptophan in breast milk compared to 2g tryptophan (p=0.008), 20g and 40g alpha-lactalbumin and no supplement group (p<0.005). The 2g tryptophan group had significantly higher free tryptophan in breast milk compared to no supplement, 20g and 40g alpha-lactalbumin (p<0.005) and lower levels compared to 4g tryptophan (p=0.008). The 20g alpha-lactalbumin group had significantly lower free tryptophan in breast milk values compared to 2g and 4g tryptophan group (p<0.005) and no significant difference compared to no supplement and 40g alpha-lactalbumin group (p=0.424, p=0.474 respectively). The 40g alpha-lactalbumin group had a significantly lower free tryptophan in breast milk levels compared to 2g and 4g tryptophan group (p<0.005) and no difference compared to no supplement and 20g alpha-lactalbumin group (p=0.136, p=0.474 respectively).

There was a significant effect of group on the AUC of free tryptophan in breast milk (F (4,25)=20.86, p<0.001). The Post hoc LSD test revealed that the 4g l-tryptophan group had a significantly higher AUC of free tryptophan in breast milk compared to 2g l-tryptophan (p=0.008), 20g and 40g alpha-lactalbumin and no supplement group (p<0.001). The 2g l-tryptophan group had significantly higher AUC of free tryptophan in breast milk compared to no supplement, 20g and 40g alpha-lactalbumin (p<0.005) and lower levels compared to 4g l-tryptophan (p=0.008). The 20g alpha-lactalbumin group had significantly lower AUC of free
tryptophan in breast milk values compared to 2g and 4g l-tryptophan group ($p<0.005$) and not different from no supplement and 40g alpha-lactalbumin group ($p=0.466$, $p=522$ respectively). The 40g alpha-lactalbumin group had a significantly lower AUC of free tryptophan in breast milk levels compared to 2g and 4g l-tryptophan group ($p<0.005$) and not different from no supplement and 20g alpha-lactalbumin group ($p=0.177$, $p=0.522$ respectively).

Peak concentration of free tryptophan in breast milk after 2g and 4g tryptophan doses was around 2 hours and then started to decrease. This peak was very minimal for 40g and 20g alpha-lactalbumin doses and was observed around 4 hours and then was almost back to baseline levels after 6 hours.

After administration of tryptophan or alpha-lactalbumin, plasma tryptophan and free tryptophan in breast milk were highly correlated, particularly with samples taken at the same time, or with samples in which the plasma sample predated the breast milk sample by one to two hours ($r=0.459$ to 0.781, $p=0.11$ to $<0.001$).
Repeated measures analysis of variance showed a strong effect of group ($F(12,75)=13.64$, $p<0.005$) Timing was identical for all groups. The data has been staggered for easier viewing. Error bars represent standard deviation.
3.4.4. Free Tryptophan Levels in Infant Formula

Twelve different marketed North American infant formulas (6 random samples from each brand) were measured for their free tryptophan contents. Formulas were chosen both from hydrolyzed and non-hydrolyzed types. Two infant formulas had free tryptophan contents below the limit of detection (<0.01mg/100ml) (Isomil-Soy Protein Steps 1 and 2) and in other 10 the free tryptophan ranged from 0.64-510.4 mg/100 ml prepared formula across the samples (Figure 3-5). All study groups had significantly lower levels of free tryptophan in breast milk compared to free tryptophan in extensively hydrolyzed formulas (Nutramigen A+ and Alimentum) (p<0.005). There were no significant differences between any other formulas and our supplemented group.
Figure 3-5. Free Tryptophan in Common Infant Formulas and in Breast Milk after Oral Tryptophan or Alpha-lactalbumin (a-lac) Supplementation.

To capture the variation in free breast milk levels, the y axis was broken to 3 segments. Mean and standard deviation are presented. Free tryptophan in breast milk even with the highest dose of 4g tryptophan was comparable to non-hydrolyzed formulas, and significantly lower than extensively hydrolyzed formulas (Nutramigen A+ and Alimentum) (analysis of variance, post-hoc comparisons, Fisher's least significant difference (LSD), $p<0.005$)
3.5. Discussion

This is the first study to assess whether oral tryptophan or proteins rich in tryptophan affect tryptophan levels in breast milk. We found no effect of oral tryptophan or alpha-lactalbumin on total tryptophan levels in breast milk despite elevations in plasma free tryptophan. Free tryptophan levels in breast milk, which represented 2% of the total levels, rose, but the free levels were comparable to what is found in several common infant formulas. This result, while explainable, is distinct from other pharmacological aids for sleep.

A plausible explanation why oral supplementation with tryptophan or alpha-lactalbumin did not affect total tryptophan levels in breast milk is that free tryptophan constitutes only a small proportion of the total tryptophan in breast milk. The relative timing of the rise in free tryptophan in blood plasma with the subsequent rise in free tryptophan in breast milk can be accounted for by rapid transfer of tryptophan from the former to the latter. As shown in Figure 3-2, free tryptophan in plasma peaked 1 to 2 hours after oral supplementation, and the free tryptophan in breast milk, as shown in Figure 3-3, peaked 2 to 4 hours after oral supplementation. As would be expected with a transfer of free tryptophan from plasma to breast milk, the rise in free tryptophan levels in breast milk exhibited a dose-response relationship. The strong correlation between free tryptophan in plasma and breast milk is consistent with passage of tryptophan from plasma to the free tryptophan compartment of breast milk (i.e. the 1 to 2 per cent of total tryptophan in breast milk). Although free levels of tryptophan in breast milk were affected, it is known that 95% to 99% of amino acids in breast milk are contained within proteins and peptides with only a small portion being free amino
acids (Svanberg et al. 1977). This is consistent with the present study in which, on average, after tryptophan supplementation, 98% of tryptophan was in peptides and proteins and only 2% was free tryptophan. With only 2% of total tryptophan influenced by free plasma tryptophan levels in breast milk, the effect of influencing free tryptophan levels in breast milk upon total levels becomes negligible as was observed (Figure 3-1).

The absence of an effect of oral tryptophan upon total tryptophan concentration in breast milk paves the way to investigating tryptophan as part of dietary supplement in clinical trials for postpartum sleep disturbances. Most investigations of tryptophan in healthy subjects demonstrates tryptophan efficacy with mild insomnia, and primarily for decreasing sleep latency, improving sleep, and increased total sleep time (Fernstrom 2012; Silber and Schmitt 2010). Oral tryptophan may also increase slow-wave sleep (SWS) and reduce rapid-eye movement (REM) sleep (Fernstrom 2012). Other pharmacological aids for sleep such as lorazepam, diazepam, and zopiclone demonstrate milk/plasma ratios of 0.1 to 2.7 across individuals after single oral dosing (Briggs et al. 2011), which contrasts with no change (equivalent of zero) for tryptophan.

The lack of effect of oral tryptophan upon total tryptophan levels in breast milk also supports further investigation of tryptophan as part of a dietary supplement strategy to reduce depressed mood in early postpartum. Severity of sad mood during the early postpartum blues, which peak on day 5 on average, is highly predictive of later clinical level PPD (Adewuya 2006; O'Hara and Swain 1996). Since tryptophan levels may influence mood (Fernstrom 2012; Young et al. 1985), there is potential to attenuate severity of depressed during peak postpartum blues as a strategy to reduce subsequent risk of PPD. Serotonin synthesis in the brain is dependent on transport of plasma tryptophan through the LNAA
transporter, where it is converted to 5-hydroxytryptophan and subsequently via tryptophan hydroxylase to serotonin. Consistent with this, empirical studies show that increasing or decreasing plasma tryptophan influences serotonin levels in brain homogenates (Gessa et al. 1974; Moja et al. 1989; Wurtman and Fernstrom 1975). This strategy is further supported by the finding that MAO-A levels are increased in women by over 40% during day 4 to 6 postpartum, and that MAO-A is the primary metabolic route of serotonin, so tryptophan may have potential as a replacing supplement over this time (Sacher et al. 2010). Attenuating effects of high MAO-A levels has potential for preventing MDE because elevated MAO-A levels occur in the prefrontal and anterior cingulate cortex during MDEs, postpartum MDEs, and during high risk states for MDE such as prior to recurrence of illness, early postpartum, perimenopause, and exposure to toxicities that create risk for MDE such as heavy alcohol and cigarette abuse (Bacher et al. 2011; Johnson et al. 2011; Meyer et al. 2006; Meyer et al. 2009; Rekkas et al. 2014; Sacher et al. 2010; Sacher et al. 2011).

The concentration of free tryptophan in breast milk after oral supplementation was similar to free tryptophan levels in non-hydrolyzed and partially hydrolyzed formulas and significantly lower than extensively hydrolyzed infant formulas. Interestingly, this is also the first study that measured free tryptophan in multiple random samples of popular infant formulas in North America. Previous reports of free amino acids in infant formulas evaluated 1 random sample in each of 8 different brands in North America (Ventura et al. 2012) and 1 sample per 18 different brands available in Spain (Alegria et al. 1999); however their results, from a non-statistical perspective, have a similar rank of effect (amongst extensively hydrolyzed, partially hydrolyzed and non-hydrolyzed formulas) to our statistically supported observations.
Tryptophan has been helpful with mild insomnia and decreasing latency to fall asleep, but has not been investigated for sleeping difficulties specifically in postpartum. This study supports further investigation of tryptophan for sleeping difficulties in postpartum because of the lesser effect on breast milk in contrast to most medications. We also view this study as a step for inclusion of tryptophan as part of a dietary supplementation strategy to prevent PPD. However, evidence from clinical trials of a dietary supplementation strategy is required before tryptophan can be considered for preventing PPD.

Overall the results of this study show that a single oral dose of tryptophan or alpha-lactalbumin, does not increase the total tryptophan in breast milk, and even though oral supplementation increases the free tryptophan levels in breast milk, these levels are within the range found in popular marketed infant formulas. Since a large pool of tryptophan in breast milk is contained in proteins and peptides, elevations in free tryptophan in breast milk did not increase the total tryptophan levels in breast milk. The lack of effect upon total tryptophan in breast milk opens the possibility of investigating tryptophan or proteins rich in tryptophan for their potential to improve sleep in postpartum and/or as an aid to prevent PPD.
Chapter 4  Quantitating States to Predisposition to Mood Symptoms in Postpartum

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4.1. Preface

A limitation in the field of postpartum blues is that a quantitative state measurement of severity has not been optimally developed for quantitating mood symptoms. In order to assess the efficacy of the supplement on severity of postpartum blues and to develop a novel therapeutic, there was a need for a measurement to quantify the state severity of postpartum blues. Therefore, we proposed to use mood induction procedure (MIP) and also observe its relationship to other typically used measures such as Edinburgh Postnatal Depression Scale (EPDS). For the purposes of a more theoretical academic publication, which differs in some respects with the practical aim of developing a supplement to prevent postpartum MDE, issues of novelty related to the theory of major depression were emphasized which led to an additional focus on dysfunctional attitude in increasing susceptibility to mood symptoms in postpartum. However, there are several key aspects of this publication that link tightly into the thesis directions: First there is a strong correlation between the degree of effect of the MIP towards depressed mood and EPDS score. Second, the magnitude of effect of the MIP was very strong at day 5 postpartum compared to other groups, suggesting that this measure is very sensitive to detecting mood change at day 5 postpartum.
4.2. Abstract

Purpose: Postpartum depression (PPD) is the most common complication of childbearing with a 13% prevalence. Vulnerability to depressed mood has an important role in the onset of major depressive episodes (MDE), but has not been investigated in postpartum. The aim is to assess whether day-5 postpartum blues and severity of dysfunctional attitudes predicts vulnerability to depressed mood.

Methods: 45 healthy women were recruited: Group 1 (n=12) were day-5 postpartum during the typical peak of postpartum blues. Group 2 (n=11) were within 18 months postpartum and reported a vulnerability to cry (and had elevated dysfunctional attitudes but no MDE). Group 3 (n=11) were within 18 months postpartum and no vulnerability to cry. Group 4 (n=11) were not recently postpartum. Vulnerability to depressed mood was measured by the change in the visual analogue scale from the sad mood induction procedure (MIP).

Results: Univariate analysis of covariance demonstrated that day-5 postpartum blues and level of dysfunctional attitudes were highly predictive of change in sad mood (postpartum blues: F(1,41)=12.9, p<0.005, dysfunctional attitudes scale score: F(1,41)=11.49, p<0.005).

Conclusion: Two factors (day-5 postpartum, and severity of dysfunctional attitudes) predicted vulnerability to sad mood. Since severity of postpartum blues, predicts PPD, MIP on day-5 postpartum represents a quantitative measure that can be applied to screen novel, early interventions for preventing PPD. Interventions to prevent PPD through increasing resilience against mood induction should target postpartum women with greater severity of dysfunctional attitudes.
4.3. Introduction

Postpartum depression (PPD) is the most common complication of childbearing with a prevalence rate of approximately 13% (O'Hara and Swain 1996) and an optimal approach for widespread prevention has not been established. It is generally accepted that vulnerability to sad mood is an important factor in the onset of major depressive episodes (MDE), but vulnerability to sad mood has not been explored in postpartum. In this study vulnerability to sad mood is examined in relation to several factors: dysfunctional attitudes, state of postpartum blues (day 5 postpartum) and general postpartum state (within 18 months postpartum).

The relationship of vulnerability to sad mood with dysfunctional attitudes was chosen because a cornerstone of the cognitive model of depression is that dysfunctional attitudes interact with negative life events to create depressed mood and more pessimistic dysfunctional attitudes are associated with greater risk for MDE (Alloy et al. 2006; Otto et al. 2007). While it might be assumed that greater severity of dysfunctional attitudes would be associated with greater vulnerability to depressed mood, this specific relationship has not been studied during the postpartum period. Investigations of mood induction in relation to dysfunctional attitudes have typically focused upon inducing sad mood in subjects such as in remitted and recovered depressed individuals and measuring change in dysfunctional attitudes afterwards rather than vice versa (Jarrett et al. 2012; Miranda and Persons 1988; Segal et al. 2006).
Vulnerability to sad mood is implicated in early postpartum specifically at day 5, but also thereafter. The rationale for day 5 is that it is the peak point of postpartum blues, a time of healthy range sadness reported in as many as 75% of women (O'Hara et al. 1991a; O'Hara et al. 1991b). A laboratory based method to assess the severity of postpartum blues has not been previously applied, instead, a common approach to measure postpartum blues is the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al. 1987), which is less precisely oriented towards the day of assessment, and is less state dependent, inquiring about recent insomnia, fatigue, poor appetite, crying, anxiety and emotional lability. In addition, as demonstrated by endorsement on the crying question of the EDPS, approximately 20% of women have crying spells in their first 18 months of postpartum without experiencing a full MDE (Evans et al. 2001).

Mood-induction procedures (MIP) in healthy subjects offer the potential for identification of mechanisms which may play a role in the development of clinical depression. The aim of this study was to develop a method for quantification of postpartum blues using MIP. Mood induction procedures have been used extensively in a variety of disciplines to induce a temporary mood state in human volunteers. Mood states induced include elation, depression, anger and disgust (Blin et al., 1993). One such procedure is the Velten MIP (Velten, 1968), which involves reading a set of self-referent statements describing the mood that is intended to be induced (either elation or depression for the original procedure), and asking the participant to ‘try and feel the mood suggested’. Following its initial development, this method has been used in many modified versions (Williams, 1984; Seibert and Ellis, 1991), which mainly vary from the original in the length or type of language/construct of statements used. The Velten MIP is the one of the most commonly used procedures (for a review see
Kenealy, 1986), and has been classified as highly effective for depressed mood induction (Gerrards-Hesse et al., 1994). Increasingly popular is the musical MIP, which involves the use of music to encourage development of the intended mood state, either with or without additional instructions to ‘try to feel the mood suggested’ by the music. The musical MIP may have the advantage of a greater percentage of participants responding to it compared to the Velten where 30–50% participants may fail to respond (Clark, 1983). MIP in healthy subjects offers the ability for recognizing mechanisms which may play a role in the development of depression which would ultimately lead to the testing in clinical populations.

To obtain a maximal percentage of responders to mood induction, we applied a combination of the Velten and the musical MIP.

The objective of this study was to determine whether higher dysfunctional attitude scale severity, presence of day 5 postpartum blues, or being within 18 months postpartum will be associated with greater sadness after sad mood induction. Given the importance of dysfunctional attitudes in the cognitive theory of MDE (Alloy et al. 2006; Otto et al. 2007), given that day 5 postpartum is associated with postpartum blues (O'Hara et al. 1991a); and that MDE occurs with a high prevalence in postpartum (O'Hara and Swain 1996), it is hypothesized that these three factors will be associated with greater vulnerability to negative mood induction.
4.4. Methods

4.4.1. Participants and Study Design

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada. Written informed consents were obtained from all subjects after a through explanation of the study details and each subject was free to withdraw anytime during the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki (1997).

Women aged 18 to 45 were recruited through advertisement. They were eligible to participate if they were healthy, medication-free, and not taking any investigational products. Exclusion criteria were: Currently pregnant (as screened with urine pregnancy test), use of any investigational medicinal product or herbal medication within the previous 8 weeks, diagnosed with any axis 1 and/or axis 2 disorders based on structured clinical interview (SCID) for DSM-IV, substance abuse (screened with urine drug test). In addition, to reduce variability in mood attributable to cigarette withdrawal, subjects who reported cigarette smoking in the past five years were excluded from the study.

In order to assess effects of postpartum blues, dysfunctional attitudes and general postpartum state (i.e. within 18 months postpartum), four groups of subjects were recruited for this study: Group 1 were women at day 5 postpartum. Group 2 were women within 18 months of giving birth that reported crying spells but did not have symptoms of a MDE. Since a priori the levels of dysfunctional attitudes were unknown in the subjects sampled, it was anticipated
that self report of crying spells would be more likely to sample women with higher levels of dysfunctional attitudes. Group 3 were women within 18 months of giving birth without having any crying spells and group 4 were not recently postpartum (i.e. more than 4 years since giving birth).

The protocol involved 2 visits. The first was a screening visit for eligibility and the second was the experiment day. During the second visit, subjects first completed the Stein’s Blue scale, and then underwent a neutral MIP, followed by the administration of the Visual Analog Scale (VAS), the Dysfunctional Attitude Scale (DAS), the Profile of Mood Sate (POMS) and the VAS. After a break they underwent the sad MIP and the questionnaires were repeated in the same order again. Following a second break, neutral MIP was administered again to remove any effects of sad MIP. This was followed by administering the VAS and the Beck Depression Inventory Scale (BDI).

4.4.2. Measures

The outcome measures used in this study were: 1) 40-item version of dysfunctional attitude scale (DAS). Form A and form B of the DAS were administered in a counterbalanced design on the second visit to additionally assess stability of the DAS. Dysfunctional Attitude Scale was administered before and after the sad MIP (DAS 1 and DAS 2). DAS form A was also administered at screening (DAS 0) 2) the 10-point scale visual analog scale (VAS) with 8 items consistent with how subjects feel in the moment. The items included depressed, happy, restless, sad, anxious, angry, drowsy and alert (Kendell et al. 1981). Change scores were
measured during the sad MIP. 3) 65 adjective version of profile of mood state (POMS). Six factors are derived that include tension, depression, anger, fatigue, vigor and confusion (McNair et al. 1971). Change scores were measured during the sad MIP. 4) Additional measures included the Stein’s blue scale for symptoms of postpartum blues, which consists of 13 symptoms evaluated at the moment (depression, crying, anxiety, tension, restlessness, exhaustion, dreaming, appetite, headache, irritability, poor concentration, forgetfulness and confusion) and the 10-item Edinburgh postnatal depression scale (EPDS) (Cox et al. 1987) to detect symptoms of PPD over the past 7 days.

### 4.4.3. Mood Induction

Two forms of mood induction were applied: neutral and sad mood induction. To induce sad and neutral mood states the Velten (Velten 1968) MIP was used in combination with the approach of Clark et al (Clark 1985). The Velten method is the most widely used technique for studying affective influences upon behavior and it has demonstrated effectiveness in altering subjective emotional states (Frost & Green, 1982). Velten MIP is a series of 60 self-referent statements. Negative statements reflected pessimism, dissatisfaction, and lethargy; for example “life is a heavy burden”. Neutral statements example was such as “an orange is a citrus fruit”. Subjects were asked to read each statement, printed individually, first to themselves and then aloud, and to ‘feel and experience each statement as it would apply to you personally’. In addition, to facilitate the MIP, participants were also presented with a piece of music (while reading the statements), from work by Clark et al (Clark 1985). For sad
MIP, subjects listened to Prokofiev’s “Russia under the Mongolian Yoke” and for neutral MIP, they listened to Mozart’s “Piano Concerto No. 21 in C Major”.

### 4.4.4. Statistical Analyses

First, stability of the DAS on the main experiment day was assessed across the mood conditions for the four groups applying repeated measures of Analysis of Variance (ANOVA). DAS pre-mood induction (DAS 1) and DAS post-mood induction (DAS 2) were dependent variables, and group and DAS order were between subject factors.

The main analysis was a univariate analysis of covariance (ANCOVA) with change in depressed mood measured with the VAS as the dependent variable, and predictor variables of DAS 1 score (covariate), presence of day 5 postpartum blues, and being within 18 months postpartum (between subject factors). In addition, the same analysis was completed applying change score of depression subscale of the POMS as the dependent variable.

In addition a univariate analysis of variance (ANOVA) was done with change in VAS mood scores as dependent variable and group as between subject factor. The same analysis was completed applying the change in depression subscale of the POMS as the dependent variable.
4.5. Results

Forty five healthy women completed this study (Figure 4-1). Of these, 12 women were in group 1 (day-5 postpartum), 11 were in group 2 (within 18 months postpartum, report vulnerability towards crying but not experiencing a MDE), 11 were in group 3 (within 18 months postpartum with no vulnerability towards crying), and 11 were in group 4 (not recently postpartum) (Table 4-1). The mean age was similar across the four groups.

Table 4-1. Clinical Demographic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD or %</td>
<td>N=12</td>
<td>N=11</td>
<td>N=11</td>
<td>N=11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>32.75±3.39</td>
<td>29.09±4.18</td>
<td>31.18±4.17</td>
<td>31.09±6.74</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>Committed Relationship*</td>
<td>100%</td>
<td>81.8%</td>
<td>90.9%</td>
<td>18.2%</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Level of Education &gt;</td>
<td>High School</td>
<td></td>
<td></td>
<td></td>
<td>0.162</td>
<td></td>
</tr>
<tr>
<td>Concomitant Medication</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity, Caucasian</td>
<td>75%</td>
<td>45.4%</td>
<td>54.5%</td>
<td>36.4%</td>
<td>0.298</td>
<td></td>
</tr>
</tbody>
</table>

* Committed relationship referred to being married or common-law

Group 1: day-5 postpartum women; Group 2: Women within 18 months postpartum, report vulnerability towards crying but not experiencing a major depressive episode; Group 3: Women within 18 months postpartum with no vulnerability towards crying; Group 4: Women not recently postpartum
Figure 4-1. Subject Recruitment Detail
Repeated measures of ANOVA revealed a slight increase in DAS scores (towards pessimism) after depressed mood induction (F (1,44)=5.87, p=0.02) with an 6.3 point rise in DAS scores on average, but no effect of order (i.e. form A or form B first) (F (1,43)=1.14, p=0.29), or group (F (3,41)=0.85, p=0.47).

Univariate analysis of covariance with change in VAS depressed mood scores (before and after sad MIP) as a dependent variable, found that both baseline dysfunctional attitudes score and being day 5 postpartum predicted the change in VAS depressed mood scores (effect of dysfunctional attitudes, (F (1,41)=11.49, p<0.005); effect of day 5 postpartum, (F (1,41)=12.9, p<0.005)). More specifically, greater severity of dysfunctional attitudes and being day 5 postpartum both predicted greater change in VAS towards depressed mood after mood induction (Figure 4-2). While we prioritized the DAS administered at the baseline of the day of mood induction, similar results were observed in this model with the use of any DAS scores (screening, before sad MIP or after sad MIP. Being within 18 months of pregnancy (but not day 5 postpartum) did not influence result of the MIP measured by the change in depressed mood scores on VAS, but there was a trend (F (1,41)=3.54, p=0.07).

Similarly, ANCOVA with the change in POMS/depression scores (before and after sad MIP) as a dependent variable, found that both baseline dysfunctional attitudes score and being day 5 postpartum both predicted the change in POMS/depression scores (effect of dysfunctional attitudes, (F (1,41)=6.31, p=0.01); effect of day 5 postpartum, (F (1,41)=6.45, p=0.01)). Again, being within 18 months of pregnancy (but not day 5 postpartum) did not influence result of the MIP measured by the change in POMS/depression scores (F (1,41)=1.83, p=0.18).
Figure 4-2. Significant Correlation between Dysfunctional Attitude Scale (DAS) Scores Prior to Mood Induction and the Change in Depressed Mood Scores on Visual Analog Scale (VAS)

R=0.630, p<0.005
Recruitment group was also assessed as a predictor of change in depressed mood on the VAS. ANCOVA showed a significant group effect on change in VAS mood scores ($F(3,41)=22.45, p<0.005$) (Figure 4-3). Fisher's least significant difference (LSD) post-hoc revealed that change in VAS scores is significantly higher in group 1 compared to group 2 ($p=0.03$), group 3 ($p<0.005$), and group 4 ($p<0.005$). Change in VAS group in group 2 was significantly greater compared to group 3 ($p<0.005$) and group 4 ($p<0.005$). There was no significant difference between groups 3 and 4 ($p=0.81$). Likewise, recruitment group was also assessed as a predictor of change in POMS/depression scores. ANOVA revealed a significant effect of group on change in POMS/depression scores ($F(3,41)=8.58, p<0.005$).

In addition, to evaluate group differences in DAS 1 and DAS 2, rather than the change in DAS scores, a multivariate analysis of variance (MANOVA) was performed with DAS 1 and DAS 2 as dependant variable and group as fixed factor. Both DAS 1 ($F(3,41)=8.24, p<0.005$) and DAS 2 ($F(3,41)=8.51, p<0.005$) were significantly different among groups. LSD post-hoc revealed that both DAS 1 and DAS 2 scores was significantly higher in women in the first 18 months postpartum with crying spells ($p<0.005$). There were no significant differences in DAS 1 and DAS 2 among the other groups.
Figure 4-3. Stronger Elevation in Depressed Mood Scores in Visual Analog Scale (VAS) in Day-5 Postpartum Women

Univariate analysis of covariance showed a significant group effect on the change in VAS mood scores after the sad mood induction procedure ($F(3,41)=22.45$, $p<0.005$)
In addition, there was a significant correlation between the DAS 1 and EPDS ($r=0.55, p<0.005$). Moreover, ANOVA with change VAS depressed mood score (after depressed mood induction) as a dependent variable, and predictor variables of presence of day 5 postpartum blues, and EPDS showed that both were predictive of change in VAS (EPDS: $F(1,42)=24.29, p<0.005$; day 5: $F(1,42)=4.85, p=0.03$).

4.6. Discussion and Conclusion

This study has several novel findings: Greater dysfunctional attitude levels and being day 5 postpartum predicted vulnerability to depressed mood, and cognitive reactivity was present in this healthy sample. The predisposition to mood induction has implications for developing resilience against depressed mood in postpartum through psychological and biological means, an important issue, given that PPD is the most common adverse complication of childbearing and that, according to the world health organization, MDE is the leading cause of death and disability in women aged 18 to 44.

The sadness of day 5 postpartum is considered in the healthy range of experience, however, when postpartum blues is more severe it is associated with a much higher likelihood of PPD (Henshaw et al. 2004; O'Hara et al. 1991b). Hence precise quantitation of postpartum blues could be applied as an improved screening procedure to assess whether novel interventions, that are candidates for reducing risk of PPD, create resilience against PPB. Interventions that create resilience against PPB could then be prioritized as interventions to assess for prevention of PPD. For example, this approach could screen for benefit of dietary
supplements proposed to prevent PPD, such as omega 3 fatty acids, and amino acid precursors for monoamines (Mozurkewich et al. 2013; Sacher et al. 2010): Such supplements often involve combinations of ingredients, so individual ingredients or combinations thereof which successfully prevent PPB in small scale trials could be brought forward in larger scale clinical trials to assess whether they also prevent PPD. In addition it is likely that additional concepts for dietary supplements will emerge since several mechanisms have been proposed to explain sadness in early postpartum such as elevated MAO-A levels (Sacher et al. 2010), neuroinflammation, inadequate GABA(A)R delta-subunit plasticity (Maguire and Mody 2009), decreased neurogenesis (Pawluski and Galea 2007), and reductions in brain derived neurotrophic factor signaling (Suda et al. 2008).

Most studies of mood induction and cognitive reactivity focused upon the relationship of mood induction to change in DAS; this paper focuses upon the effect of DAS upon vulnerability to depressed mood in women, most of whom are postpartum. To the best of our knowledge, this is the first study to empirically demonstrate a relationship between greater DAS and greater vulnerability to depressed mood, a finding cardinal to the cognitive development of major depressive episodes. Interestingly, in the present study there was also an overall effect of MIP that raised the severity of the DAS, across women, in all groups. To the best of our knowledge, studies have not focused on healthy women with no past MDE (Gemar et al. 2001). Cognitive reactivity can be viewed as a marker of risk for onset of later MDE in those with MDD who do not receive cognitive behavioural therapy (Jarrett et al. 2012; Miranda and Persons 1988; Segal et al. 2006), and a marker not reported in previous samples of healthy individuals of both sexes. Since both mood induction leads to greater severity of DAS and greater DAS leads to greater severity of depressed mood consequent to
the mood induction, this provides support for the possibility that both effects may sequentially and repetitively contribute to the development of depressed mood. Presently CBT is applied as a treatment for full MDE, and prevention of recurrence in postpartum, but it would be an interesting future direction to consider applying some elements of this approach for prevention in otherwise healthy women who have high DAS scores in early postpartum.

Our study is the first to show that DAS scores are elevated in women complaining of increased tendency to cry in the first 18 months postpartum. While crying itself, represents a more mild symptom, high DAS scores are an important psychological target because greater DAS scores are associated with greater hopelessness which is associated with greater risk for suicide (Beck et al. 1989; Beck et al. 1985). Women with psychiatric symptoms in postpartum are at more than 10 fold greater risk for suicide (Comtois et al. 2008). The phenomenon of crying without a full MDE is present in large datasets but the etiology has been unclear: In a sample of 9000 women who completed the Edinburgh Depression Inventory, at 8 weeks and 8 months post delivery, Evans et al reported that approximately 10% of women report symptom severity compatible with a MDE, and an additional 20% of women endorse crying episodes (Evans et al. 2001). The mechanism for the elevated DAS scores in this group is unclear since DAS influenced by development, personality, and presence of past MDE (Butler et al. 2002; Segal et al. 2006). Anecdotally, 24% women in the present study reported the tendency to cry as new onset and not present prior to giving birth, suggesting that this might have been acquired. In conclusion, this study suggests that two separate conditions of being in immediate postpartum and having high dysfunctional attitudes during postpartum are predictive of vulnerability to sad mood. The vulnerability to
sad mood during postpartum blues could be applied as a screening procedure for new candidate methods to prevent PPD. Interventions that suppress the mood induction of PPB would also be more likely create resilience against PPD since the former is associated with risk for the latter. Not only was greater DAS is associated with greater vulnerability to depressed mood but it was also shown that mood induction itself raises the DAS during postpartum. This raises the possibility that during postpartum, events which induce sad mood, can lead to greater DAS thereby reinforcing further sad mood. Finally this is the first study to identify greater DAS in healthy women who cry more than usual. Methods to identify women likely to have elevated DAS in routine clinical settings are important because this symptom is linked to hopelessness and suicide, the latter being a high risk problem in postpartum when substantial psychiatric symptoms are present.
Chapter 5  Effect of a Dietary Supplement on Predisposition to Depressed Mood in Postpartum: An Open-Label Trial
5.1. Abstract

**Purpose:** Although postpartum depression (PPD) is the most common complication of childbearing with a 13% prevalence rate, there are currently no widespread prevention strategies for PPD. Severity of postpartum blues (PPB) predicts the risk of postpartum depression (PPD). One approach in developing a strategy for preventing PPD is to develop a supplement to prevent PPB. As elevated levels of monoamine oxidase-A (MAO-A) have been reported during PPB, particularly in the prefrontal and anterior cingulate cortex, it is hypothesized that a dietary supplement based on monoamine precursors and antioxidants may have promising results in preventing PPD. The specific aim of this open-label study was to assess whether a dietary supplement consisting of tryptophan (2g), tyrosine (10g) and blueberry extract/juice can reduce the intensity of PPB at day-5 postpartum, the typical peak of PPB.

**Methods:** 26 healthy women were recruited and assigned into 2 groups: Control group (n=12) were day-5 postpartum not receiving any supplements, Supplemented group (n=14) were day-5 postpartum, receiving the dietary supplements. Severity of PPB was measured as the vulnerability to depressed mood quantitated by the change in the visual analogue scale mood scores (VAS) and Profile of Mood State depression scores from the sad mood induction procedure (MIP).

**Results:** Univariate analysis of variance (ANOVA) demonstrated a significantly greater change in depressed mood on the VAS in controls compared to supplemented group (F
Similarly, ANOVA revealed a significant effect of group on change in POMS/depression scores ($F(1,24)=25.31, p<0.001$).

**Conclusion:** In this open trial, administration of this dietary supplement composed of 2g tryptophan, 10g tyrosine and blueberry extract/juice virtually eliminated the intensity of PPB. This supports further investigation of the dietary supplement in a double blind, randomized, placebo controlled trial, to reduce PPB, as the next phase, in order to assess whether this combination can prevent PPD.

5.2. **Introduction**

Postpartum blues (PPB), also known as baby blues or maternity blues, occurs in approximately 75% of women (O’Hara et al. 1991a; O’Hara et al. 1991b). Postpartum blues is a mild syndrome involving fatigue, insomnia, poor appetite, crying, anxiety and emotional lability. It is usually seen within days 4 to 6 postpartum, with the peak on day 5 (Harris et al. 1994). Symptoms usually resolve within 10 days; however, some individuals advance to postpartum depression (PPD) (Kendell et al. 1981; O’Hara et al. 1991a; O’Hara et al. 1991b). Severity of postpartum blues, particularly on day 5, is predictive of risk for PPD (Adewuya 2006; Hannah et al. 1992; O’Hara et al. 1991b); hence it is plausible that interventions that decrease postpartum blues may prevent PPD.

Greater MAO-A levels are implicated in the etiology of postpartum blues. Data from a human positron emission tomography (PET) study shows that early in postpartum after the initial estrogen decline, during the healthy range of postpartum blues, monoamine oxidase A
(MAO-A) VT, an index of MAO-A density, is elevated by 43% throughout the brain (Sacher et al. 2010). This includes subregions of the prefrontal and anterior cingulate cortex, structures with abnormal function in major depressive disorder (Ressler and Mayberg 2007). It has also been reported that MAO-A VT, is elevated with a similar regional distribution in human brain during major depressive episodes (MDE) and prior to recurrence of MDE (Johnson et al. 2011; Meyer et al. 2006; Meyer et al. 2009). MAO-A levels are associated with MAO-A activity and there are particular functions of MAO-A which, if increased, are implicated in depressed mood. MAO-A metabolizes serotonin, norepinephrine and dopamine and creates hydrogen peroxide (H₂O₂), a pro-oxidant (Youdim and Bakhle 2006). Depletion of these neurochemicals is associated with sad mood. Oxidative stress is associated with anxiety behaviours in rodents and markers of oxidative stress are sometimes elevated in mood disorders (Bortolato et al. 2008; Bouayed et al. 2009; Brown et al. 2014; Choy et al. 2010).

It might be possible to compensate for the effects of elevated MAO-A levels and activity by the use of dietary supplement. Tryptophan and tyrosine are brain penetrant monoamine precursors which could be applied to compensate for excessive monoamine metabolism. Administration of blueberry juice and blueberry extract might also be helpful due to their anthocyanin content since anthocyanins have anti-oxidant properties (Wu et al. 2006). Some reports suggest that several anthocyanins contained in blueberries are brain penetrant (Andres-Lacueva et al. 2005; Joseph et al. 2003; Kalt et al. 2008), which is consistent with their molecular weight. The rationale for a combination of these ingredients is to counter elevated MAO-A levels in early postpartum and it is hypothesized that a dietary supplement consisting of the monoamine precursors tryptophan and tyrosine, and antioxidants may be
effective in reducing the intensity of postpartum blues.

The present study aimed to determine the effect of a dietary supplement composed of tryptophan and tyrosine along with blueberry juice/extract on reducing the intensity of sadness in postpartum blues. In a previous study, it has been shown that oral tryptophan and tyrosine supplements do not increase their total concentrations in breast milk (Dowlati et al. 2014a) (Chapter 3). This is most likely attributable to the issue that in breast milk, approximately 98% of these amino acids are predominantly found in proteins and peptides. These proteins and peptides would not be expected to be affected by oral supplementation of the individual amino acids, as was found. At doses of 10g of tyrosine and 2g of tryptophan, the supplements were able to significantly increase their free concentrations in maternal plasma, which could be predicted to eventually lead to higher transport to the brain, yet did not affect the total concentration of these amino acids in breast milk. For measuring vulnerability to sadness, the mood induction procedure (MIP) was used (Clark et al. 1999; Gemar et al. 2001). Greater vulnerability to sadness occurs during PPB (Dowlati et al. 2014b).
5.3. Methods

5.3.1. Participants and Study Design

This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada and the Natural Health Product Directorate and Drug Directorate of Health Canada. Written informed consent was obtained from all subjects after a thorough explanation of the study and each subject was free to withdraw anytime during the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki (1997).

Women aged 18 to 45 were recruited through advertisements. They were eligible to participate if they were healthy, medication-free, and not taking any investigational products. Exclusion criteria were: use of any investigational medicinal product or herbal medication within the previous 8 weeks, current diagnosis or history of any axis 1 and/or axis 2 disorders based on structured clinical interview (SCID) for DSM-IV, or substance abuse (screened with urine drug test). In addition, to reduce variability in mood attributable to cigarette withdrawal, subjects who reported cigarette smoking in the past five years were excluded from the study.

Subjects were recruited and screened during the last trimester of pregnancy and were randomly assigned to one of the 2 groups: Control group were women on day-5 postpartum not receiving the dietary supplement. Supplemented group were women on day-5 postpartum receiving tryptophan, tyrosine and blueberry extract/ juice. Control group was presented in a previous study (Dowlati et al. 2014b). The protocol involved 2 visits. The
first visit was a screening visit for eligibility which occurred during the last trimester of pregnancy and the second visit was the active protocol day which occurred on day-5 postpartum. After the initial screening (visit 1), the intervention group received a combination of the supplement ingredients over the course of 3 days, starting 2 days prior to the second visit. The timing of supplement intake is shown in Table 5-1. Tryptophan and tyrosine supplements were given at different time points, in order to avoid competition between the two amino acids for transport though the blood-brain-barrier by Large Neutral Amin Acids (LNAA) Transporters.

Table 5-1. Design and Timing of Dietary Supplement Intake

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night of Day 3 Postpartum</td>
<td>Blueberry Drink Intake (Blueberry extract + Blueberry juice)</td>
</tr>
<tr>
<td>Morning of Day 4 Postpartum</td>
<td>Blueberry Drink Intake (Blueberry extract + Blueberry juice)</td>
</tr>
<tr>
<td>Night of Day 4 Postpartum</td>
<td>2g Tryptophan Tablets + Blueberry Drink Intake (Blueberry extract + Blueberry juice)</td>
</tr>
<tr>
<td>Morning of Day 5 Postpartum (Active Protocol Day)</td>
<td>10g Tyrosine Capsules + Blueberry Drink Intake (Blueberry juice + Blueberry extract)</td>
</tr>
</tbody>
</table>
During the second visit, subjects underwent a neutral MIP, followed by the administration of the visual analog scale (VAS), the profile of mood state (POMS) and the VAS. After a 5 minutes break they underwent the sad MIP and the questionnaires were repeated in the same order again. Following a second break, neutral MIP was administered again to remove any effects of sad MIP. This was followed by administering the VAS and the Beck Depression Inventory Scale (BDI) and EPDS.

The dietary supplements consisted of 2g tryptophan, 10g tyrosine, blueberry extract and blueberry juice. These optimal doses were selected in accordance to previous studies demonstrating that they do not increase total tryptophan and total tyrosine in breast milk, significantly elevate free tryptophan and free tyrosine in plasma and do not cause any side effect (Dowlati et al. 2014a)(Chapter 3). Tyrosine capsules were obtained from Trophic Canada (Trophic Canada, Richmond Hill, Ontario, Canada, NPN#80010004). Each capsule contained 500mg L-tyrosine. The L-tryptophan capsules were obtained from Apotex and (Apo-tryptophan by Apotex Incorporated, Toronto, Ontario, Canada, DIN# 02248539). Each capsule contained 1g of tryptophan. Apo-tryptophan and Trophic L-tyrosine have been approved for use in Canada by Health Canada. The product monograph and investigator brochure for these products may be found at Health Canada’s website. The blueberry extract powder was obtained from Future-Ceuticals and called VitaBlue (FutureCeuticals, Momence, Illinois, USA). Blueberry juice and blueberry extract have been prepared and packaged by Guelph Food Technology Centre (GFTC). Blueberry extracts were packaged in sachets containing 1g blueberry extract each. Blueberry juice was obtained from Milne Fruit Products and packaged by Guelph Food Technology Centre (GFTC) in 280 millilitres bottles. At the point of consumption, the blueberry juice and extract were intended to mix to form a
readily digestible beverage. The reason blueberry extract has been added to the blueberry juice is that in the process of pasteurization of the blueberry juice under high temperatures, many of the anthocyanins are damaged (Buckow et al. 2010). Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacity in the food industry. The ORAC assay measures the degree of inhibition of peroxyl-radical-induced oxidation by the compounds of interest in a chemical milieu (Wang et al. 1996). Consistent with the issue of anthocyanin susceptibility to heat degradation, the mean ORAC for blueberry beverage alone was 5.95 TE/g and for the blueberry beverage mixed with the blueberry extract was 18.75 TE/g.

5.3.2. Measures

The primary outcome measure used in this study was the change in the 10-point scale visual analog scale (VAS) for rating depressed mood after MIP. The VAS set had a total of 7 items consistent with how subjects feel in the moment. The additional items included ratings for being drowsy, excited, tense, stressed, without energy, and anxious (Kendell et al. 1981). Average VAS scores were calculated for the two VASs obtained prior to sad MIP and for the 2 VAS obtained after sad MIP and the difference between these averages represented the change score. As an additional measure of sadness, the 65 adjective version of profile of mood state (POMS) was also applied at the same times as the VAS. From the POMS, six factors are derived that include depression, tension, anger, fatigue, vigor and confusion (McNair et al. 1971). Change scores attributable to the MIP were calculated in the same manner as the VAS. Another measure that was applied at baseline on day 5 was the 10-item
Edinburgh postnatal depression scale (EPDS) (Cox et al. 1987) to detect symptoms of PPD over the past 7 days (but subjects were asked to apply this to the past 5 days).

5.3.3. Mood Induction

Two forms of mood induction were applied: neutral and sad mood induction. To induce sad and neutral mood states the Velten (Velten 1968) MIP was used in combination with the approach of Clark et al (Clark 1985). The Velten method is the most widely used technique for studying affective influences upon behavior and it has demonstrated effectiveness in altering subjective emotional states (Frost & Green, 1982). Velten MIP is a series of 60 self-referent statements. Negative statements reflected pessimism, dissatisfaction, and lethargy; for example “life is a heavy burden”. Neutral statements example is such as “an orange is a citrus fruit”. Subjects were asked to read each statement, printed individually, first to themselves and then aloud, and to ‘feel and experience each statement as it would apply to you personally’. In addition, to facilitate the MIP, participants were also presented with a piece of music (while reading the statements) from work by Clark et al (Clark 1985). For sad MIP, subjects listened to Prokofiev’s “Russia under the Mongolian Yoke” and for neutral MIP, they listened to Mozart’s “Piano Concerto No. 21 in C Major”.
5.3.4. Statistical Analyses

The primary analysis was univariate analysis of variance (ANOVA) with the change in average VAS mood scores before and after sad MIP as the dependant variable and group (control or supplemented group) as a between subject factor. The secondary analysis was ANOVA with the change in depression subscale of the POMS as the dependant variable and group (control or supplemented group) as between subject factor.

5.4. Results

Twenty four subjects completed the study (Figure 5-1). Of these, 12 women were in control group (day-5 postpartum not receiving dietary supplement), 12 were in supplemented group (day-5 postpartum receiving dietary supplement). The mean age was similar in two groups being, 32.75±3.39 and 31.58±5.12 respectively. Demographics and clinical characteristics of each group are presented in Table 5-2.
Figure 5-1. Subject Recruitment Detail

Unable to Contact = 3

Inquired About the Study = 34

Day-5 Postpartum Contacted and Assessed for Eligibility = 33

Not Eligible = 6
Not Interested = 6

Provided Consent = 19

Dropout = 3
Not Eligible = 2

Eligible After Full Screening = 14

Completed Day-5 Postpartum Supplement Group = 14

Completed Control Group = 12
(Day-5 Postpartum, Refer to Fig. 4-3)
Table 5-2. Clinical Demographic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Supplemented Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=12</td>
<td>N=14</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>32.75±3.39</td>
<td>31.58±5.12</td>
<td>0.381</td>
</tr>
<tr>
<td>Committed Relationship*</td>
<td>100%</td>
<td>93%</td>
<td>0.365</td>
</tr>
<tr>
<td>Primiparous</td>
<td>58.3%</td>
<td>50%</td>
<td>0.686</td>
</tr>
<tr>
<td>Level of Education &gt; High School</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, Caucasian</td>
<td>75%</td>
<td>78.6%</td>
<td>0.838</td>
</tr>
<tr>
<td>Concomitant Medication</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Family/Friend Support</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Neonatal Complications</td>
<td>0%</td>
<td>7.1%</td>
<td>0.365</td>
</tr>
<tr>
<td>Obstetrical Complications</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Multivitamin Intake</td>
<td>91.6%</td>
<td>100%</td>
<td>0.289</td>
</tr>
</tbody>
</table>

* Committed relationship referred to being married or common-law
Supplementation was assessed as a predictor of change in depressed mood on the VAS. ANOVA showed a significant group effect on change in VAS mood scores (F (1,24)=229.46, \( p<0.001 \)) \textbf{(Figure 5-2)}. The results demonstrated that receiving the dietary supplement was significantly associated with minimal or no change in mood as measured by the VAS mood scores after sad mood induction. VAS depressed mood score change after the sad MIP was 56.5±9.41 in controls and 1.75±8.99 in supplemented group. The effect size was 5.9.

Likewise, recruitment group was also assessed as a predictor of change in POMS/depression scores. ANOVA revealed a significant effect of group on change in POMS/depression scores (F (1,24)=25.31, \( p<0.001 \)) \textbf{(Figure 5-3)}. POMS/depression score change after the sad MIP was 13±9.07 in controls and was -0.07±3.29 in supplemented group.
Figure 5-2. Stronger Elevation in Depressed Mood Scores in Visual Analog Scale (VAS) in Day-5 Postpartum Women not Taking the Dietary Supplement.

Analysis of variance found significant elevation in depressed mood scores as measured by VAS in day-5 postpartum women not taking any supplements compared to day-5 postpartum women taking the dietary supplement (F (1,24) = 229.46, p < 0.001).
Figure 5-3. Stronger Elevation in Depression Scores in Profile of Mood State (POMS) in Day-5 Postpartum Women not Taking the Dietary Supplement.

Analysis of variance found significant elevation in depression scores as measured by POMS in day-5 postpartum women not taking any supplements compared to day-5 postpartum women taking the dietary supplement ($F(1,24)=25.31, p<0.001$).
5.5. Discussion

This is the first study to investigate the effect of a combination of monoamine precursors, tryptophan and tyrosine, and blueberry extract/juice on the intensity of postpartum blues. The results of this study illustrate that the dietary supplement, consisting of 10g tyrosine, 2g tryptophan and blueberry juice/extract, was able to reduce the intensity of postpartum blues on day 5 postpartum as measured by sad MIP. These results need to be considered in the context of an open trial, but are very substantial and hold greater promise than previous supplement studies in early postpartum.

There was a large effect associated with the supplement condition. Being an open trial, this effect could be attributable to a combination of active effect and placebo effect. It is plausible that some level of belief that the dietary supplements might be helpful may have reduced the induction of depressed mood. However, it is highly unlikely that the result observed is solely due to placebo because the effect size in this study on severity of postpartum blues being 5.9 is much greater than interventions in other open trials, which would also include placebo effects. For example, Chabrol et al. randomly assigned 37 women in their third trimester to 3 groups of: providing information on PPB, providing and discussing information on PPB and control (no intervention) (Chabrol et al. 2007). The effect size for comparison of mean scores on the EPDS completed between 3rd and 5th days postpartum was 0.07 to 0.28 across groups. Another study randomly assigned 169 women on their second or third postpartum day to information group (receiving written information about PPB and PPD) or control group and evaluated them with EPDS on day 3, week 3, and
3 months postpartum. The percentage of scores above 11 in the EPDS around birth was 8.5% in the information group versus 9.3% in the control group, which again reflects a modest effect size (Kleeb and Rageth 2005).

One explanation to account for an active effect is that the supplement is compensating for the effects of monoamine metabolism and increased oxidative stress by elevated MAO-A level. A second reason to ascribe an active effect is that each ingredient also demonstrated some efficacy in relevant contexts. Previous human studies have demonstrated that 100-150 mg/kg/day of tyrosine reduce adverse mood and performance impairments due to environmental stresses (Banderet and Lieberman 1989; Deijen and Orlebeke 1994; Glaeser et al. 1979; Melamed et al. 1980; Shurtleff et al. 1994; Stone 1975; Yeghiayan et al. 2001). Most investigations of tryptophan in healthy subjects have reported improved sleep, increased total sleep time and reduced waking time (Fernstrom 2012; Silber and Schmitt 2010). There was one negative result of a double-blind placebo controlled randomized control trial (RCT) which found no effect of tryptophan at a daily dose of 3g for the first 10 days postpartum on PPB or PPD symptoms, although it is possible that compliance was low since no aspect of the tryptophan intake was monitored (Harris 1980). To the best of our knowledge there has only been one study investigating the combination of tryptophan and tyrosine on mood: When tyrosine (100 mg/kg) was added to tryptophan (5g per day) for four weeks, antidepressant effects were observed in ten cases of major depressive episodes (Van Praag 1990). Future study in a randomized double blind placebo controlled trial will be necessary to differentiate the contribution of the active effect.

The results of the present study, albeit in an open trial, reflect by far the most robust effects of a dietary supplement on postpartum blues. The other evaluation of a dietary supplement
for which we are aware assessed in a randomized, placebo controlled trial the effect of 220 mg docosahexanoic acid (DHA), 220 mg each DHA and arachidonic acid (AA) or placebo from week 16 of pregnancy until 3 months postpartum, and found no statistically significant difference between groups as measured by the blues questionnaire within one week postpartum in 60 subjects (Doornbos et al. 2009). Moreover, this study focused on measuring the effect of the dietary supplement on the intensity of postpartum blues at one-day time point. Future study should evaluate whether the benefits of the dietary supplement will persist or whether additional supplements would be useful.

The results of the study demonstrated a profound effect of the dietary supplement on postpartum blues. The effectiveness of the supplement must be tempered with the issue that this was an open trial. Nevertheless, with an effect size of 5.9, there is excellent reason to pursue this supplement in a randomized double blind placebo controlled trial to further assess its effects on postpartum blues.
Chapter 6   General Discussion, Study Limitations, Future Directions
6.1. General Discussion

Collectively the projects of this thesis support continued development of a dietary supplement consisting of monoamine precursors, tryptophan and tyrosine, and blueberry juice/extract for preventing mood symptoms in postpartum. While the initial arguments for investigating this combination were theoretical, the individual projects have supported continued inclusion of the ingredients in the combination. The results of different chapters of this thesis supported the initial hypotheses proposed.

There is a logical theoretical rationale for why amino acid supplementation should not affect total levels of the amino acid in breast milk; though this had not been empirically tested before. It would be expected that amino acids freely cross into breast milk with different variability similar to medications. However, in contrast to medications, such crossing should not affect total concentrations of amino acids such as tryptophan and tyrosine into breast milk. The reason is that approximately 98% to 99% of tryptophan and tyrosine in breast milk is contained in proteins and peptides and only about 1% to 2% is considered as free tyrosine and free tryptophan (Lemons et al. 1983; Macy 1949; Svanberg et al. 1977; Wurtman and Fernstrom 1979). Oral supplement would be expected to influence free amino acids levels but not the vast majority of these amino acids when contained in the fixed chain sequences of proteins and peptides.

Even though the theory was compelling, given that these supplements were planned for administration to breastfeeding women, it was vital to verify this. The results from the first two studies, described in chapters 2 and 3, illustrated that oral tryptophan and oral tyrosine
did not elevate total tryptophan and total tyrosine concentrations in breast milk (Figures 2-1 and 3-1). The effect of oral tyrosine and oral tryptophan upon their levels in breast milk and plasma had not been previously examined in breastfeeding mothers. Free levels of these amino acids did rise in breast milk (Figures 2-3 and 3-3); however they were within the range reported in popular marketed infant formulas and significantly lower than extensively hydrolyzed infant formulas (Figures 2-4 and 3-4). Maternal plasma tyrosine and tryptophan significantly increased with the magnitude of the oral tyrosine or oral tryptophan supplements (Figures 2-2 and 3-2), demonstrating that, first, there was enough change in plasma level to potentially influence monoamine levels in maternal brain, and second, that the lack of effect on total tryptophan or tyrosine levels in breastmilk is not attributable to an inadequate level of change in the plasma concentrations of these amino acids. Significant positive correlations between free tryptophan or tyrosine in plasma with breast milk is consistent with passage of tryptophan or tyrosine from plasma to the free tryptophan or tyrosine compartment of breast milk (i.e. the 1 to 2 per cent of total tryptophan and total tryptophan in breast milk).

There had been a shortcoming in the literature of a state dependent postpartum blues scale. This is of significant importance, given that the other scales measured mood symptoms over longer periods of time, with most covering a week. In addition, postpartum blues as a phenomenon reflects responsiveness to the environment, which was not phenomenologically captured by the previous scales and they lack the ability to measure all the negative aspects of mood symptoms in postpartum. We discovered a sensitive state measure of vulnerability to depressed mood in postpartum which also correlated with previous scales such as EPDS. We were able to observe the significant shift towards sadness in day-5 postpartum women.
compared to non-postpartum after the sad mood induction as measured with the visual analog scale (VAS) mood scores (Figure 4-2). Similar results were observed in women during 18 months with vulnerability towards crying as compared to same groups of women without vulnerability towards crying. The sensitivity of the mood induction on day 5 suggests its utility as a development tool, which could be applied for a number of approaches to prevent PPD. This new quantification method was then used in the open trial study, described in chapter 5.

Among doses investigated for oral tyrosine (chapter 2), 10g dose was chosen to be investigated in the next study as it was highly tolerated by the mothers and produced the highest elevation in maternal plasma. As for oral tryptophan doses (chapter 3), the 2g dose was chosen since some subjects receiving the 4g experienced nausea. Therefore, we finally investigated the effect of a dietary supplement composed of tryptophan and tyrosine and blueberry juice/extract in an open trial. The results showed that the dietary supplement combination significantly attenuated the change in sad mood induction as measured by the mood scores on VAS (Figure 5-1). We discovered that women on day-5 postpartum who received the dietary supplement were significantly feeling less sad after the sad MIP compare to women on day-5 postpartum not receiving any supplements, with the average change of mood scores measured by VAS being 56.5±9.4 and 1.42±9.71 respectively, showing an effect size of approximately 5.9. It can be understood that it was empirically the combination of the dietary supplement that yielded positive results in the open trial.

The rationale behind the development of dietary supplement combination investigated in this thesis is based on the high MAO-A levels in early postpartum. MAO-A oxidatively metabolizes neurotransmitters serotonin, norepinephrine and dopamine (Ou et al. 2006;
Youdim et al. 2006). After the initial estrogen decline in early postpartum, MAO-A levels and activity are elevated by over 40% in affect controlling regions such as the prefrontal and anterior cingulate cortex, during the healthy range postpartum blues. Moreover, MAO-A levels are elevated during MDEs in these regions, as well as during high risk states for MDE such as prior to recurrence of illness, early postpartum, perimenopause, and exposure to toxicities that create risk for MDE such as heavy alcohol and cigarette abuse (Bacher et al. 2011; Johnson et al. 2011; Meyer et al. 2006; Meyer et al. 2009; Rekkas et al. 2014; Sacher et al. 2010; Sacher et al. 2011). Given the neurobiological model of high MAO-A levels in early postpartum, a key strategy to lower the intensity of postpartum blues, was to counter the acute rise in MAO-A and loss of multiple monoamines. MAO-A levels are highly correlated with MAO-A activity and to counter elevated MAO-A levels in early postpartum tryptophan (precursor to serotonin), tyrosine (precursor to dopamine and norepinephrine), and antioxidants should be helpful. Moreover, each monoamine precursor investigated, has mostly shown positive effects on mood and stress. Previous human studies have revealed that tyrosine doses ranging 100-150 mg/kg/day were able to reduce adverse mood and performance impairments due to environmental stresses (Banderet and Lieberman 1989; Deijen and Orlebeke 1994; Glaeser et al. 1979; Melamed et al. 1980; Shurtleff et al. 1994; Stone 1975; Yeghiayan et al. 2001). Most investigations of tryptophan in healthy subjects have also reported the supplements ability to improve sleep, increase total sleep time and reduced waking time (Fernstrom 2012; Silber and Schmitt 2010), and may also increase SWS and decrease REM sleep (Fernstrom 2012). In addition, tryptophan levels may influence mood (Fernstrom 2012; Young et al. 1985). Monoamine metabolism by MAO-A is oxidative and pro-oxidant states cause anxiety in animals; hence, an antioxidant should be
useful. Therefore there is potential to attenuate severity of depressed mood during peak postpartum blues as a strategy to reduce subsequent risk of PPD. While many foods and fruits contain antioxidants (Halvorsen et al. 2006), blueberries were chosen for their antioxidant content, as they contain a number of different anthocyanins in contrast to other fruits which have mainly cyanidin anthocyanins (Table 1-1) (Wu et al. 2006). A number of the anthocyanins, especially, malvidin and cyanidin anthocyanins have been reported to be detectable in the brains of blueberry fed rodents and pigs, and blueberry administration is associated with resistance to cognitive decline in rodents, suggesting again that the contents are brain penetrant (Andres-Lacueva et al. 2005; Joseph et al. 2003; Joseph et al. 1999; Joseph et al. 1998; Kalt et al. 2008).

Overall, since the severity of postpartum blues is associated with higher risk of PPD, a method of preventing postpartum blues is greatly needed. Reducing the intensity of postpartum blues may assist with reducing the incidence of PPD. While broad widespread prevention strategies are lacking for PPD, there is a good rational for developing a dietary supplements consisting of monoamine precursors tryptophan and tyrosine and blueberry juice/extract based on the theory of high MAO-A in early postpartum. In conclusion, there were a number of empirical findings in the projects included in this thesis that confirms progressing with the dietary supplement combination proposed for preventing PPD.
6.2. Study Limitations

Few limitations can be mentioned for the studies performed. For the first study described in chapter 2 a limitation is that we were not able to calculate the T-max and half-life for free tyrosine in breast milk for some of the oral tyrosine doses. However, for the maximum dose of 10 g oral tyrosine we were able to detect the peak and subsequent decrease in free tyrosine levels in breast milk and total tyrosine levels in breast milk did not exhibit any change with any or the oral tyrosine doses. In addition it should be noted that the plasma levels in mothers were near the baseline levels for most doses, specially the maximum dose, at the end of the protocol. As mothers had to bottle feed their infant for the purpose of this study, we chose this duration that they were most comfortable and agreeable with. Moreover, length of stay had to be considered as an important factor as they had to attend the laboratory for the purpose of the study.

A limitation of the fourth study described in chapter 5 is the limitation inherent to the open trail; however the magnitude of effect is so strong that it seems unlikely to be fully accounted for by the placebo effect. Moreover, this study focused on measuring the effect of the dietary supplement combination on the intensity of postpartum blues at one-day time point. Future study should evaluate whether the benefits of the dietary supplement will persist or whether additional supplements would be useful. In addition, while the focus of the fourth study was on the postpartum blues for the purpose of intervention in this thesis, it would have been interesting to further follow-up the subjects in order to inquire about PPD. Follow-up phone interviews with SCID, EPDS and BDI was done at 1 month and 3 months postpartum in the group receiving the dietary supplement and only 1 subject had developed PPD at 3 months.
However, follow-up data on the control group, not receiving the dietary supplement, was not obtained in order to compare, which may be considered a limitation.

It should be noted that to date, there have been no studies investigating the effect of blueberry manipulation on any behavioural or cognitive changes in human. The rationale for proposing blueberry as part of the dietary supplement strategy was based on few observations: high antioxidant content of blueberries (Wu et al. 2006), number of the anthocyanins being reported to be detectable in the brains of blueberry fed rodents and pigs, and blueberry administration being associated with resistance to cognitive decline in rodents (Andres-Lacueva et al. 2005; Joseph et al. 2003; Joseph et al. 1999; Joseph et al. 1998; Kalt et al. 2008). However there is no data on blueberry or antioxidants in general for mood disorders in contrast to the substantial evidence that amino acids may influence mood. Future clinical studies are warranted to assess the effects of blueberry extract upon human brain and cognition.
6.3. Implications for Future Studies

The projects described were the important steps in establishing whether a dietary supplement combination can reduce the intensity of postpartum blues and have minimal effects on breast milk. Postpartum blues intensity is associated with an increased risk of developing PPD; therefore, a dietary supplement that reduces severity of postpartum blues might also be able to reduce the risk of PPD. Currently there are no widespread standard prevention strategies for PPD. Future studies are feasible in two main directions. One important direction is to evaluate later phase studies in postpartum blues. The other direction would be to carry this forward for preventing PPD. There are few reasonable steps that we propose for future. First, in order to demonstrate specificity of the supplement versus a non-specific group of amino acids, it would be helpful to rule out in open trial whether a protein dietary supplement (which does not follow the logical design of the test supplement) influences severity of postpartum blues. Demonstrating specificity is a common step in assessing dietary supplements. For example, in studies investigating the effect of tryptophan, alpha-lactalbumin or other protein sources on emotions or cognitive functions, casein has been used as a placebo or control condition (Markus et al. 2008; Scrutton et al. 2007; Verschoor et al. 2010). Second, another issue is to assess the meaningfulness of the dose of the dietary supplement given. For example, examining the effect of half strength of this supplement will also assist in understanding the potential of the combinations proposed in reducing the intensity of postpartum blues.
Moreover, it would be useful to conduct a study evaluating whether the benefits of the dietary supplement combination will persist beyond the one-day time point. Also, investigating whether additional supplements would be useful or not is an interesting point to consider when conducting future trials with the proposed dietary supplement.

The next logical step would be examining the effect of this dietary supplement in reducing the intensity of postpartum blues in a double blind RCT. If the outcome was not entirely successful at some point, there is also the possibility of adding other ingredients. Subsequently, if the dietary supplement shows promising results in preventing postpartum blues in the RCT, the step afterwards is to perform a double blind RCT in order to investigate the effect of the dietary supplement combination in preventing PPD. If the results of the final proposed study show promising effect in the ability of the dietary supplement for PPD prophylaxis, this would be indeed the first approach in developing a new method of preventing PPD in women who are otherwise healthy.

One important future direction is in regards to women at high risk for developing PPD, whom at the moment have none or minimal symptoms of PPD (i.e. having past history of MDE). History of MDE is considered a moderate to strong risk factor for PPD (effect size of 0.38-0.39) (Beck 2001). For this group, who are asymptomatic but at high risk, antidepressants would usually be considered but compliance is typically low. Also, there is issue of antidepressant exposure to infant through breastfeeding. Since common practice shows that many mothers want absolutely minimal exposure, assessing the effect of a dietary supplement for efficacy in this group of women is of significant value. Another issue is that the strategy of prophylaxis is largely based on the prophylactic effect of preventing MDE (which is substantial). Evidence for PPD alone, specifically, is less compelling and a recent
meta-analysis suggests more data is needed to argue a better case for evidence of antidepressant prophylaxis of PPD (Howard et al. 2005).

Another potential future direction relates to treatment of subclinical symptoms of PPD. It has been proposed that normalization of MAO-A levels in postpartum is conducive towards achieving a healthy mood state (Sacher et al. 2010). With an inadequate decline of MAO-A, one can develop PPD or later develop postpartum crying symptoms (without having PPD). As mentioned in earlier sections, there is approximately 14% elevation in MAO-A binding, particularly in prefrontal cortex and anterior cingulate cortex of women with crying spells due to sad mood in early postpartum (but not having full symptoms of MDE) (Sacher et al. 2015). MAO-A was also elevated in regions of high MAO-A density such the hippocampus and in regions that have been implicated in mood disorders. Therefore, in order to counter elevated MAO-A levels in women with subsyndromal mood symptoms in postpartum it is also possible that a dietary supplement consisting of monoamine precursors and antioxidants may also be effective in reducing this sadness.

Although the use of the proposed dietary supplement in this thesis is for immediate postpartum, but the results presented in this thesis can pave the way of testing the proposed dietary supplement in other conditions with high MAO-A levels (Table 6-1). One condition is perimenopause, which is a period of high risk for mood disorders, and it has been proposed to be a window of risk for processes linked to later dementia. Results of a recent PET study has shown that there is on average, 34% elevation in MAO-A $V_T$ in perimenopausal age compared with reproductive age and by 16% compared with menopause (Rekkas et al. 2014). Therefore assessing new intervention strategies to prevent these changes can prevent mood changes and reduce long-term risk of neurodegenerative illness. Elevations in MAO-A $V_T$, is
also observed in the prefrontal and anterior cingulate cortex during other sad/dysphoric states such as early alcohol withdrawal and early cigarette withdrawal, which have potential for being targeted effectively with this supplement. People with alcohol dependence regularly experience sadness and dysphoria during early alcohol withdrawal (APA 2000; Bokstrom et al. 1989). Subjects with alcohol withdrawal have on average 32% elevation in MAO-A $V_T$ in different brain regions. MAO-A $V_T$ in these regions was correlated with severity of depressed mood (Matthews et al. 2014). In addition people who smoke cigarettes also regularly experience sadness and dysphoria during acute cigarette withdrawal (Carey et al. 1993; Kenford et al. 2002). Early cigarette withdrawal is associated with 25% elevation and in MAO-A $V_T$ in prefrontal and anterior cingulate cortex compare to healthy controls. Also, the magnitude of rise in MAO-A $V_T$ in these regions was significantly correlated with the shift in the mood scores on visual analogue scales towards depressed mood (Bacher et al. 2011).
Table 6-1. High Monoamine Oxidase-A States

<table>
<thead>
<tr>
<th>Condition State</th>
<th>Control Group</th>
<th>Average Brain MAO-A Elevation (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum Blues (N=15)</td>
<td>Healthy women not recently pregnant (N=15)</td>
<td>43%</td>
</tr>
<tr>
<td>(Sacher et al. 2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First onset PPD (N=15)</td>
<td>Healthy women not recently pregnant (N=15)</td>
<td>21%*</td>
</tr>
<tr>
<td>(Sacher et al. 2015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postpartum crying due to sad mood (without symptoms of MDE) (N=12)</td>
<td>Asymptomatic postpartum healthy women (N=15)</td>
<td>14%</td>
</tr>
<tr>
<td>(Sacher et al. 2015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women of perimenopausal age</td>
<td>Women of reproductive age</td>
<td>34%</td>
</tr>
<tr>
<td>(N=27) (Rekkas et al. 2014)</td>
<td>(N=19)</td>
<td></td>
</tr>
<tr>
<td>Early withdrawal from heavy cigarette smoking (N=24)</td>
<td>Healthy non-smoking</td>
<td>25%</td>
</tr>
<tr>
<td>(Bacher et al. 2011)</td>
<td>(N=24)</td>
<td></td>
</tr>
<tr>
<td>Early withdrawal from alcohol dependence (N=16)</td>
<td>Healthy controls (N=16)</td>
<td>32%</td>
</tr>
<tr>
<td>(Matthews et al. 2014)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDE (N=17)</td>
<td>Healthy controls (N=17)</td>
<td>34%</td>
</tr>
<tr>
<td>(Meyer et al. 2006)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MAO-A: Monoamine Oxidase A; PPD: Postpartum Depression;
* This value represents the mean of MAO-A elevation in Prefrontal Cortex and Anterior Cingulate Cortex
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Appendices
Appendix 1. Visual Analog Scale
Visual Analogue Scales

Subject ID: ____________        Date: __________________  VAS ____

Please mark on the line to indicate your feelings at the present time. Please indicate your answer by making a cross \( \times \) on the line between the two extremes at the appropriate distance.

Most Happy ........................................................................ Most Depressed

Most Energy ........................................................................ Least Depressed

Least Anxious ....................................................................... Most Anxious

Alert .................................................................................. Drowsy

Calm .............................................................................. Excited

Relaxed ............................................................................... Tense

Not Stressed ....................................................................... Stressed
Appendix 2. Profile of Mood State Scale
### Profile of Mood State

**Subject’s Initial ____________**

**Date ____________________**

**Subjects Code No. _____________**

**Directions:** Describe HOW YOU FEEL RIGHT NOW by checking one space after each of the words listed below:

<table>
<thead>
<tr>
<th>Feeling</th>
<th>Not at All</th>
<th>A Little</th>
<th>Moderately</th>
<th>Quite a Bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friendly</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tense</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Angry</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Worn Out</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Unhappy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Clear-headed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Lively</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Confused</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sorry for Things</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Shaky</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Listless</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Peeved</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Considerate</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sad</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Active</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>On Edge</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Grouchy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Blue</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Energetic</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Panicky</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hopeless</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Relaxed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Unworthy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Spiteful</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sympathetic</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Uneasy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Restless</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Unable to</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fatigued</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Helpful</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Annoyed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Discouraged</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Resentful</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Nervous</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Lonely 1 2 3 4 5
Miserable 1 2 3 4 5
Muddled 1 2 3 4 5
Cheerful 1 2 3 4 5
Bitter 1 2 3 4 5
Exhausted 1 2 3 4 5
Anxious 1 2 3 4 5
Ready to Fight 1 2 3 4 5
Good-Natured 1 2 3 4 5
Gloomy 1 2 3 4 5
Desperate 1 2 3 4 5
Sluggish 1 2 3 4 5
Rebellious 1 2 3 4 5
Helpless 1 2 3 4 5
Weary 1 2 3 4 5
Bewildered 1 2 3 4 5
Alert 1 2 3 4 5
Deceived 1 2 3 4 5
Furious 1 2 3 4 5
Effacious 1 2 3 4 5
Trusting 1 2 3 4 5
Full of Pep 1 2 3 4 5
Bad-Tempered 1 2 3 4 5
Worthless 1 2 3 4 5
Forgetful 1 2 3 4 5
Carefree 1 2 3 4 5
Terrified 1 2 3 4 5
Guilty 1 2 3 4 5
Vigorous 1 2 3 4 5
Uncertain about 1 2 3 4 5
Bushed 1 2 3 4 5
Appendix 3. Velten Mood Induction Statements
VELTEN Mood Induction Procedure

Negative Mood Statements

Each statement below has been printed in a single card and is presented to an individual in a booklet. The subject will start by reading the instruction at the beginning of the booklet first.

**Instruction:** Read each of the following statements to yourself. Then read each of the statements loudly. As you look at each card, focus your observation only on that one and concentrate on each of the statements as they are in front of you. A certain amount of time will be devoted to each statement. Your success at coming to experience this mood will largely depend on your willingness to accept and respond to the idea in each statement and to allow each statement to act upon you. Attempt to respond to the feeling suggested by each statement. Then try to think of yourself as definitely being and moving into that state. Try to experience each statement as if it was especially written for you and try to believe in each statement.

1. Today is neither better nor worse than any other day
2. I feel rather slow and sluggish now
3. Every now and then I feel so tired and depressing that I'd rather just sit than do anything
4. Sometimes I wonder whether school is all that worthwhile
5. I can remember times when everybody but me seemed full of energy
6. Too often I have found myself staring listlessly into the distance, my mind a blank, when I definitely should have been studying
7. It has occurred to me more than once that study is basically useless, because you forget almost everything you learn anyway
8. People annoy me; I wish I could be by myself
9. I've had important decisions to make in the past, and I've sometimes made the wrong ones
10. I do feel somewhat discouraged and drowsy - maybe I'll need a nap when I get home
11. Perhaps college takes more time, effort, and money than it's worth
12. I just don't seem to be able to get going as fast as I used to
13. I couldn't remember things well right now if I had to
14. Just a little bit of effort tires me out
15. I've had daydreams in which my mistakes kept occurring to me - sometimes I wish I could start over again
16. I'm ashamed that I've caused my parents needless worry
17. I feel terribly tired and indifferent to things today
18. Just to stand up would take a big effort
19. I'm getting tired out. I can feel my body getting exhausted and heavy
20. I'm beginning to feel sleepy. My thoughts are drifting
21. At times I've been so tired and discouraged that I went to sleep rather than face important problems
My life is so boring - the same old thing day after day depresses me
There have been days when I felt weak and confused and everything went miserably wrong
I can't make up my mind; it's so hard to make simple decisions
I want to go to sleep - I feel like just closing my eyes and going to sleep right here
I'm not very alert; I feel listless and vaguely sad
I've doubted that I'm a worthwhile person
I feel worn out. My health may not be as good as it's supposed to be
It often seems that no matter how hard I try, things still go wrong
I've noticed that no one seems to really understand or care when I complain or feel unhappy
I'm uncertain about my future
I'm discouraged and unhappy about myself
I've lain awake at night worrying so long that I hated myself
Things are worse now than when I was younger
The way I feel now, the future looks boring and hopeless
My parents never really tried to understand me
Some very important decisions are almost impossible for me to make
I feel tired and depressed; I don't feel like working on the things I know I must get done
I feel horribly guilty about how I've treated my parents at times
I have the feeling that I just can't reach people
Things are easier and better for other people than for me. I feel like there's no use in trying again
Often people make me very upset. I don't like to be around them
It takes too much effort to convince people of anything. There's no point in trying.
I fail in communicating with people about my problems
It is so discouraging the way people don't really listen to me
I've felt so lonesome before, that I could have cried
Sometimes I've wished I could die
My thoughts are so slow and downcast. I don't want to think or talk
I just don't care about anything. Life just isn't any fun
Life seems too much for me - my efforts are wasted
I'm so tired
I can't concentrate or move. I just want to forget about everything
I have too many bad things in my life
Everything seems completely pointless and empty
I feel dizzy and weak. I need to put my head down and not move
I don't want to do anything
I hate my life. I wish I had a different life
Everything I do is useless
All of my unhappiness of my past life is taking possession of me
I want to go to sleep and never wake up
VELTEN Mood Induction Procedure

Neutral Mood Statements

Each statement below has been printed in a single card and is presented to an individual in a booklet. The subject will start by reading the instruction at the beginning of the booklet first.

**Instruction:** Read each of the following statements to yourself. Then read each of the statements loudly. As you look at each card, focus your observation only on that one and concentrate on each of the statements as they are in front of you. A certain amount of time will be devoted to each statement. Your success at coming to experience this mood will largely depend on your willingness to accept and respond to the idea in each statement and to allow each statement to act upon you. Attempt to respond to the feeling suggested by each statement.

1. There are 60 minutes in one hour
2. Many states supply milk for elementary school children
3. Manchester is in the United Kingdom
4. Strawberries are picked in the summer
5. Basket weaving was invented before pottery making
6. Some baseball bats are made from the wood of the ash tree
7. 99.1% of Alaska is owned by the federal government
8. It snows in Scotland
9. Perennials bloom every year
10. Arizona has both deserts and pine-covered mountains
11. You have to take the ferry to get to the island
12. London is the capital of England
13. Elephants carried the supplies
14. The Pacific Ocean has fish
15. Most secondary schools have a choir
16. The rug was made according to an old Indian pattern
17. Some think that electricity is the safest form of power
18. Most oil paintings are done on canvas
19. Many buildings in Washington were made of marble
20. Corn is sometimes called maize
21. An orange is a citrus fruit
22. Some say that ladybirds are good for the garden
23. New York City is in New York State
24. Diamonds can really cut glasses
25. Some chimps have been taught to use sign language
26. Agricultural products comprised 70% of the income
27. Some streets are still listed under their old names
28. Some kinds of apple are red
29. This desk is very old
30. It is warmer in the summer
31. The names on the Christmas mailing list are alphabetically ordered
32. The doorkeeper was dressed in red
33. A free sample will be given to each person who enters the store
34. There are 3 months in every season
35. You can pick peaches in the summer
36. This building has few floors
37. California is in the west coast of the United States
38. There are many kinds of snowflakes
39. Japan was elected to the United Nations almost fourteen years after Pearl Harbor
40. There is a large rose-growing center in Texas
41. Air Canada has many flights between Toronto and European cities
42. The ship was ancient, and would soon be retired from the fleet
43. It was their sixth consecutive best seller
44. The merges did not change the company's policy.
45. The Chinese language has few dialects, including Cantonese and Mandarin
46. There are many forests in South America
47. Canada and United States are neighbors
48. Roses come in different colors
49. The newspapers had been front-paging it for days
50. Coffee breaks are usually 15 minutes
51. The repairmen fixed the broken pipe
52. The magazine’s report was interesting
53. Maps can be used as good guides
54. Black and white pictures were arranged in different sections
55. Utah is a beehive state
56. The TV show started on time
57. It is warm and humid in Florida
58. There are 7 days in a week
59. The kids are playing in the park
60. White papers are used for the copy machine