Selective dietary supplementation in early postpartum is associated with high resilience against depressed mood

Yekta Dowlati[a,b,c], Arun V. Ravindran[a,b,c], Zindel V. Segal[d], Donna E. Stewart[b,c,e], Meir Steiner[b,c,f], and Jeffrey H. Meyer[a,b,c,1]

[a]Research Imaging Centre, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada M5T 1R8; [b]Institute of Medical Science, University of Toronto, Toronto, ON, Canada; M5S 1A8 [c]Department of Psychiatry, University of Toronto, Toronto, ON, Canada M5T1R8; [d]Department of Psychology, University of Toronto, Toronto, ON, Canada M5S 3G3; [e]Toronto General Hospital Research Institute, Toronto, ON, Canada M5G 2C4; and [f]Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, ON, Canada L8S 4L8

Edited by Marcus E. Raichle, Washington University in St. Louis, St. Louis, MO, and approved February 3, 2017 (received for review July 21, 2016)

Medical research is moving toward prevention strategies during prodromal states. Postpartum blues (PPB) is often a prodromal state for postpartum depression (PPD), with severe PPB strongly associated with an elevated risk for PPD. The most common complication of childbearing, PPD has a prevalence of 13%, but there are no widespread prevention strategies, and no nutraceutical interventions have been developed. To counter the effects of the 40% increase in monoamine oxidase A (MAO-A) levels that occurs during PPB, a dietary supplement kit consisting of monoamine precursor amino acids and dietary antioxidants was created. Key ingredients (tryptophan and tyrosine) were shown not to affect their total concentration in breast milk. The aim of this open-label study was to assess whether this dietary supplement reduces vulnerability to depressed mood at postpartum day 5, the typical peak of PPB. Forty-one healthy women completed all study procedures. One group ($n = 21$) received the dietary supplement, composed of 2 g of tryptophan, 10 g of tyrosine, and blueberry juice with blueberry extract. The control group ($n = 20$) did not receive any supplement. PPB severity was quantitated by the elevation in depressed mood on a visual analog scale following the sad mood induction procedure (MIP). Following the MIP, there was a robust induction of depressed mood in the control group, but no effect in the supplement group [43.85 ± 18.98 mm vs. 0.05 ± 9.57 mm shift; effect size: 2.9; $F(1,39) = 88.33$, $P < 0.001$]. This dietary supplement designed to counter functions of elevated MAO-A activity eliminates vulnerability to depressed mood during the peak of PPB.

postpartum blues | prodrome | prevention | monoamine oxidase A | monoamine

It is increasingly believed that the optimal approach to prevent neuropsychiatric illnesses is to target prodromal states. Postpartum blues (PPB), a subclinical syndrome of low mood and emotional lability often associated with fatigue, insomnia, poor appetite, and anxiety, may be conceptualized as a prodromal state, given that severe PPB is strongly predictive of later clinical-level postpartum depression (PPD) (1–5). For example, O'Hara et al. (2) reported a 6% risk of PPD in women without severe PPB, compared with 23% in women with severe PPB. PPB affects ∼75% of women during postpartum days 4–6, typically peaking on day 5 and resolving within 10 days (5). Most research criteria define PPD as a major depressive episode (MDE) occurring within the first 1–12 mo postpartum and identify it as the most common complication of childbearing, with an overall prevalence of 13% (6, 7). For women with a previous MDE, either antidepressants or intensive evidence-based psychological therapy may help prevent PPD (8), but for the majority of pregnant women with no history of an MDE, there is no standard, widespread approach for preventing new-onset PPD.

Given that severe PPB heralds the onset of PPD, it may be useful to decrease the severity of PPB to prevent PPD. The present study focused on a dietary supplement-based strategy to create resilience against an important component of the neurobiology of PPB. The peak of PPB at postpartum day 5 occurs just after a several hundred-fold drop in the level of estrogens and a 50-fold decline in progesterone level. Given that paradigms of simulated pregnancy involving similar changes in estrogens and progesterone levels elicit depressive symptoms, sequelae of these hormonal changes are implicated in the neurobiology of PPB (6, 9). In human studies, the most prominent brain change identified in PPB is a 43% elevation in monoamine oxidase A (MAO-A) levels, extending into affect-modulating brain regions (10–14). This elevated MAO-A level may be attributed in large part to the decline in the level of estrogens, given the known inverse relationship between changes in the concentration of

Significance

Postpartum blues is a healthy range of sadness that peaks on day 5 after giving birth in most women. Severe postpartum blues is a high-risk state for postpartum depression. A dietary supplement was designed to compensate for a temporary rise in a brain protein, monoamine oxidase A, which occurs on postpartum day 5. This study tested whether this dietary supplement reduces the sadness associated with the postpartum blues. Total levels of tryptophan and tyrosine in breast milk are not affected by this dietary supplement. Women received the dietary supplement over postpartum days 3–5 or received no supplement. This dietary supplement dramatically reduced the vulnerability to sadness on postpartum day 5, the peak of the postpartum blues, eliminating the prodrome of postpartum depression.

Author contributions: Y.D., A.V.R., Z.V.S., D.E.S., M.S., and J.H.M. designed research; Y.D. and J.H.M. performed research; Y.D. and J.H.M. contributed new reagents/analytic tools; Y.D. and J.H.M. analyzed data; and Y.D. and J.H.M. wrote the paper.

Conflict of interest statement: Y.D. is developing natural health products to overcome a high MAO-A state in early postpartum. J.H.M. has received operating grant funding for other studies from Eli Lilly, GlaxoSmithKline, Bristol-Myers Squibb, Lundbeck, SK Life Science, and Johnson & Johnson/Janssen in the past 5 years. J.H.M. has served as a consultant for all of these companies except Johnson & Johnson, as well as for Sepracor, Trius Therapeutics, and Mylan Inc. None of these companies participated in the funding, design, or execution of this study or preparation of the manuscript. J.H.M. is developing natural health products to treat high-risk states for major depressive episode, and is listed as the inventor on a patent application for this dietary supplement. J.H.M. is applying for patents to implement measures using MAO to diagnose or treat mood disorders and to use peripheral measures as surrogate measures for brain inflammation. Z.V.S. receives royalties from Guilford Press for books related to mindfulness-based cognitive therapy and fees for training workshops. He also serves on the advisory board of MindfulNoggin, part of NogginLabs, a private company specializing in customized web-based learning. D.E.S. is a member of the scientific advisory board for the Cymbalta (duloxetine) Pregnancy Registry for Eli Lilly. She is a co-author of UpToDate chapters on antidepressant drugs during pregnancy and their effects on exposed infants. A.V.R. and M.S. do not have any conflicts of interest related to this work.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: jeff.meyer@camhpet.ca.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1611965114/-/DCSupplemental.

Table 1. Demographic and clinical characteristics

Characteristic	Control group (n = 20)	Supplemented group (n = 21)	Statistical comparison
Age, y, mean ± SD	32.55 ± 3.07	31.73 ± 5.26	$F(1,39) = 0.363$, $P = 0.550$
Committed relationship, %*	90	95.2	$\chi^2(1) = 0.414$, $P = 0.520$
Primiparous, %	50	42.9	$\chi^2(1) = 0.210$, $P = 0.647$
Level of education, %			
≥4-y university	65	71.4	$\chi^2(1) = 0.196$, $P = 0.658$
>high school	100	95.2	$\chi^2(1) = 0.976$, $P = 0.323$
History of previous MDE, %	0	0	
Caucasian ethnicity, %[†]	70	81	$\chi^2(1) = 0.666$, $P = 0.414$
Concomitant medication use, %	0	0	
Body mass index, mean ± SD	26.78 ± 3.47	26.51 ± 5.37	$F(1,39) = 0.037$, $P = 0.849$
Multivitamin intake, %	95	100	$\chi^2(1) = 1.076$, $P = 0.300$
Male fetal sex, %	65	47.6	$\chi^2(1) = 1.257$, $P = 0.262$
Neonatal complications, %[‡]	0	4.7	$\chi^2(1) = 0.976$, $P = 0.323$
BDI screening, mean ± SD	5.20 ± 2.71	5.86 ± 6.11	$F(1,39) = 0.195$, $P = 0.661$
NEO PI-R neuroticism, mean ± SD[§]	73.35 ± 21.61	71.05 ± 29.23	$F(1,39) = 0.082$, $P = 0.777$
NEO PI-R extraversion, mean ± SD[§]	120.70 ± 18.58	119.48 ± 14.81	$F(1,39) = 0.054$, $P = 0.817$
NEO PI-R openness, mean ± SD[§]	118.35 ± 19.06	118.10 ± 17.41	$F(1,39) = 0.002$, $P = 0.965$
NEO PI-R agreeableness, mean ± SD[§]	126.50 ± 19.08	125.76 ± 22.15	$F(1,39) = 0.013$, $P = 0.910$
NEO PI-R conscientiousness, mean ± SD[§]	130.20 ± 18.36	131.90 ± 21.51	$F(1,39) = 0.074$, $P = 0.787$

*Committed relationship defined married or being in a committed relationship for >2 y.
[†]Ethnicity categories included Asian, South Asian, Caucasian, Hispanic, Native American, Black, Arab, Pacific Islander, and mixed ancestry.
[‡]One infant in the supplemented group had jaundice.
[§]The NEO PI-R was administered during screening.

estrogens and MAO-A mRNA, density, and activity (15–19). Reductions in brain GABA levels and increased serotonin transporter levels have also been reported in human studies, and in rodents altered sensitivity of GABA type A receptor δ subunits, reduced Ca^{2+}/calmodulin-dependent protein kinase IIα, and reduced neurogenesis in the hippocampus have been reported in the early postpartum period (20–22).

The aim of the present study was to determine whether a dietary supplement kit designed to counter the sequelae of elevated MAO-A levels in the early postpartum period reduces a woman's vulnerability to depressed mood during PPB. We hypothesized that administration of the dietary supplement would generate resiliency against the induction of depressed mood in the early postpartum period. Depressed mood and mood lability are symptoms of PPB (23). Vulnerability to the induction of depressed mood is a quantitative measure of the severity of these symptoms and also a marker of elevated risk for MDE (24, 25). The reason for prioritizing elevated MAO-A levels in the design of this supplement was that the timing of this change was definitively demonstrated during PPB and the magnitude of MAO-A elevation throughout grey matter during PPB is enormous, including brain regions that generate emotion such as the prefrontal cortex, anterior cingulate cortex, and hippocampus (13, 14). Moreover, it is well known that elevated MAO-A level is associated with MDE, depressed mood states, and high-risk states for MDE (26–29).

Because MAO-A protein density is often highly correlated with MAO-A activity in the brain (30, 31), the supplement included several ingredients designed to counter the effects of enhanced MAO-A function, including increased metabolism of serotonin, norepinephrine, and dopamine. MAO-A catalyzes the oxidative deamination of serotonin, dopamine, and norepinephrine (32). One of the products of this reaction is hydrogen peroxide (H_2O_2). With higher MAO-A activity, these levels might become supraphysiological, which may generate excessive oxidative stress, with potential adverse cellular consequences (32). Oxidative stress has been associated with anxiety behaviors in rodents, and markers of oxidative stress are sometimes elevated in mood disorders (33, 34). The serotonin precursor tryptophan was added to the supplement to compensate for excessive serotonin removal, and the norepinephrine and dopamine precursor tyrosine was intended to compensate for excessive norepinephrine and dopamine removal. To counter pro-oxidant effects, a combination of blueberry juice and blueberry extract was included, which contains the dietary antioxidants anthocyanins. Investigations of blueberry anthocyanins have suggested that they cross the blood-brain barrier, and manipulations of tryptophan and tyrosine levels in plasma through dietary challenges have been shown in some studies to affect the release of serotonin, dopamine, and, to a lesser extent, norepinephrine in rodent brain (35–39). Moreover, administering tryptophan and tyrosine in excess of dietary levels has been empirically demonstrated to have no effect on their total concentrations in breast milk, a finding consistent with longstanding knowledge that 99% of amino acids in breast milk are in fixed chains within peptides and proteins (40, 41).

Results

Fifty-two subjects were enrolled in the protocol, and seven dropped out of the study (five in the supplemented group and two controls) after their first visit. One dropout had moved away, three dropouts indicated that they did not wish to commit to the time involved, and three stopped returning phone calls. Four subjects (all from the supplemented group) were excluded, one because of a high baseline depressed mood on the visual analog scale (VAS), which could have biased her change score in favor of the study result; one who required an immediate caesarean

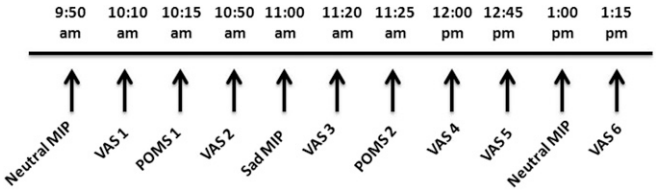

Fig. 1. Timing of the VAS and POMS relative to the MIP.

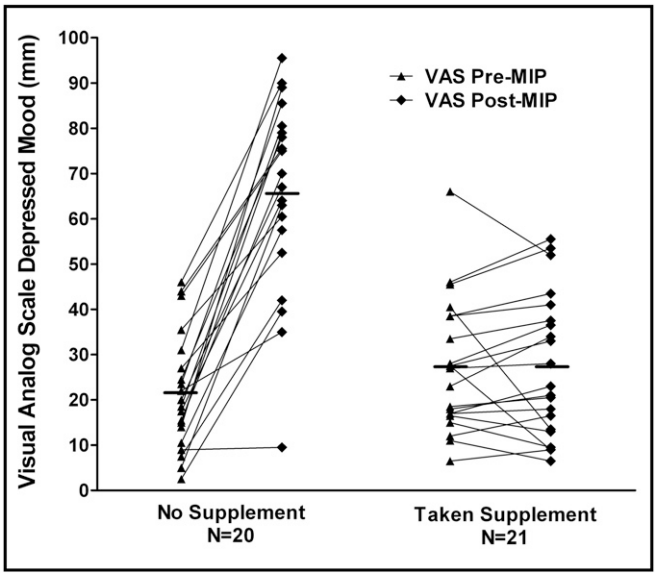

Fig. 2. Elimination of sad mood induction in postpartum women after taking the supplement. There was a highly significant elevation in depressed mood scores as measured by a VAS in day-5 postpartum women not taking any supplements compared with day-5 postpartum women taking the dietary supplement [$F(1,39) = 88.33$, $P < 0.001$, repeated-measures ANOVA].

section due to a major obstetrical complication; one who had a positive urine drug screen on postpartum day 5; and one because of an unexpected preterm delivery birth. Forty-one subjects completed the study protocol (20 controls, 21 in the supplemented group). The mean age was similar in the two groups: 32.55 ± 3.07 y in controls and 31.73 ± 5.26 y in the supplemented group. Other demographic and clinical characteristics of the two groups were similar as well (Table 1). Total Beck Depression Inventory (BDI) on screening day and postpartum day 5 and NEO Personality Inventory-Revised (NEO PI-R) subscale scores were similar in the two groups. The dietary supplement was well tolerated by all subjects, and no adverse effects were reported.

An increase in depressed mood on the VAS after the mood induction procedure (MIP) was seen in the control group, which was attenuated by administration of the supplement in the supplemented group [effect size: 2.9; interaction with group: $F(1,39)=88.33$, $P < 0.001$, repeated-measures ANOVA] (Figs. 1 and 2). After the MIP, depressed mood as measured on the VAS increased by 43.85 ± 18.98 mm in the untreated controls and by 0.05 ± 9.57 mm in the supplemented group (Fig. 2).

We also assessed the effect of the dietary supplement as a predictor of change in Profile of Mood States (POMS) depression scores. There was an increase in depressed mood on the POMS after the MIP in the control group, which was attenuated by administration of the supplement in the treatment group [interaction with group: $F(1,39) = 20.24$, $P < 0.001$, repeated-measures ANOVA] (Fig. 3). After the MIP, the POMS depression score increased by 8.95 ± 8.75 in the controls and decreased by −0.05 ± 2.67 in the supplemented group.

There were no significant effects of body mass index, third trimester depressive symptoms, parity, infant sex, self-report of recent sleep quality, or presence of neonatal complications on changes in the depressed mood VAS score or POMS depression score (Table S1). Furthermore, POMS subscale scores rose in several domains after the sad MIP in controls, but these changes were negligible in the supplemented group: confusion [$F(1,39) = 10.16$, $P = 0.003$, repeated-measures ANOVA], anger [$F(1,39) = 9.63$, $P = 0.004$, repeated-measures ANOVA], fatigue [$F(1,39) = 5.10$, $P = 0.030$, repeated-measures ANOVA], and tension [$F(1,39) = 7.16$, $P = 0.011$, repeated-measures ANOVA]. Moreover, after the sad MIP, there was an increase in VAS fatigue scores in the controls, which was attenuated in the supplemented group [$F(1,39) = 8.01$, $P = 0.007$, repeated-measures ANOVA].

Two subjects in the control group and one subject in the supplemented group subsequently endorsed the structured clinical interview (SCID) for *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (DSM-IV) symptoms of MDE within the first 3 mo postpartum [exploratory analysis: $X^2(1) = 0.550$, $P = 0.458$].

Discussion

This study demonstrates a strong beneficial effect of an intervention to reduce sadness during the peak period of PPB. The principal finding was that an open trial of a dietary supplement consisting of 2 g tryptophan, 10 g tyrosine, and blueberry juice/extract virtually eliminated intense PPB. The robust effect found suggests that the approach of creating dietary kits to target neurobiological changes in prodromal states may be a promising new avenue for nutraceutical development. The degree of the effect attributable to the intervention vs. the placebo effect must be considered, however.

Evidence-based nutraceutical development frequently stems from observed associations between dietary intake and disease prevalence, followed by clinical trials to assess the effect of dietary supplementation on disease onset. Although this approach has been helpful in some circumstances (e.g., prevention of neural tube defects in newborns through folic acid supplementation), successful nutraceutical development is rare in neuropsychiatry, most likely because the pathway for nutraceutical development to prevent neuropsychiatric illnesses is not standardized.

The approach that we took for this dietary supplement is a distinctly different strategy and represents a prototypal pathway. First, a prodromal state for PPD was prioritized. Second, a dietary kit was designed to counter a key neurobiological change in elevated MAO-A levels (>40%) during the prodromal state that extended into several brain regions involved in regulating emotion. MAO-A is also a well-proven target for antidepressants and a well-characterized human

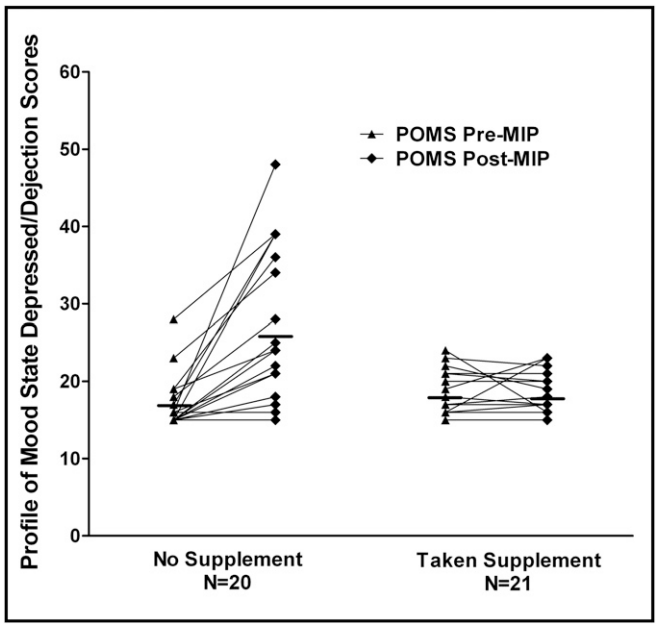

Fig. 3. No mood induction effect on POMS depression scores in postpartum women after taking the supplement. There was a significant elevation in depression scores as measured by the POMS in day-5 postpartum women not taking any supplements compared with day-5 postpartum women taking the dietary supplement [$F(1,39) = 20.24$, $P < 0.001$, repeated-measures ANOVA].

Table 2. Design and timing of dietary supplement intake

Timing	Activity
Night of postpartum day 3	Blueberry drink intake (blueberry extract and blueberry juice)
Morning of postpartum day 4	Blueberry drink intake (blueberry extract and blueberry juice)
Night of postpartum day 4	2-g L-tryptophan tablets + blueberry drink intake (blueberry extract and blueberry juice)
Morning of postpartum day 5 (active protocol day)	10-g L-tyrosine capsules + blueberry drink intake (blueberry extract and blueberry juice)

phenotype, being present in MDE, in several high-risk states for MDE onset, and in depressed mood states in both health and illness (14, 26–29). Third, evidence for brain penetrant effects was considered in the selection of individual ingredients of the dietary kit. Altering dietary tryptophan, especially by inducing a deficiency, reduces serotonin release in the prefrontal cortex of rodents and increases depressed mood in humans at high risk for MDE (36, 42). Oral tyrosine administration increases resiliency against stress-induced impairment of cognitive tasks, and depletion of tyrosine induces depressed mood and reduces stimulant-provoked dopamine release in humans and rodents (38, 39, 43, 44).

Most previous investigations in animals of blueberry-sourced anthocyanins given through the diet have reported the detection of anthocyanins in brain homogenates (35, 37). The significant effect of the supplement on the prodrome of PPB seen in the present study supports further studies to evaluate its effect on later sequelae such as PPD, with the eventual overall intent of creating a dietary supplement that may be accepted by clinicians and health care providers as a widespread approach to prevent PPD.

The main strength of this trial is its large effect size of 2.9. However, given that we conducted the first investigation of this supplement in humans with an open trial, the contribution of placebo should be considered in the context of effect size from other interventions for PPB. The magnitude of the placebo effect is widely perceived to be influenced by the level of interaction between researchers and subjects, and also by the extent of the support provided during psychoeducation interventions. In the several other intervention studies in PPB, the effect sizes on symptoms were small, suggesting that the placebo effects are also small. For psychoeducation and for dietary fish oil, effect sizes, including those for the control conditions, have ranged from 0.07 to 0.3, and the severity of PPB after these interventions was similar to the typical literature reports, suggesting that the placebo effect on PPB is low and unlikely to account for the effect size of the present study (45, 46).

This study has several limitations. The first is that this intervention to reduce PPB likely can be optimized further; for example, other biological changes in the early postpartum period, such as reduced neurogenesis, might be targeted through exercise (20), or psychosocial support could be provided concurrently to reduce stress (47). Second, although we developed the supplement with the intent of countering elevated MAO-A levels, it is possible that other mechanisms may have been responsible for the effects observed. For example, tryptophan is well known to improve sleep through reducing sleep latency, and tyrosine is known to increase resilience against stressful events (48, 49). Third, subjects were assigned based on protocol availability rather than through a fully randomized double-blind, placebo-controlled trial. The study started by measuring the sensitivity to MIP and then moved on to the effect of the proposed dietary supplement on this sensitivity. This study represents an early phase of development of a dietary supplement for preventing PPD. The results are striking, regardless of order. Finally, because the focus in this experiment was on PPB as a prodrome rather than on PPD itself, further work is needed to extend this approach to directly assess the effects on PPD.

In conclusion, a combination of orally administered tryptophan, tyrosine, and blueberry extract/blueberry juice almost completely eliminated the vulnerability to depressed mood during the peak period of PPB in our subjects, with an effect size of 2.9, which is at least 10-fold greater than the effect size reported for other interventions, including placebo. This study exemplifies a low-cost nutraceutical pathway for creating a dietary supplement kit to compensate for biological vulnerability in a prodromal state resulting in a robust reduction of symptoms.

Materials and Methods

Study Design and Study Participants. Women aged 18–45 y were recruited through advertisements and screened during the last trimester of pregnancy. These women were eligible to participate if they were healthy, medication-free, and able to provide informed consent. Exclusion criteria were use of any investigational medicinal product or natural health product medication within the previous 8 wk, current diagnosis or history of any axis 1 and/or axis 2 disorders based on the SCID for DSM-IV (50), 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 5 years were excluded from the study. The effect of the MIP was previously reported in a subset of 11 controls (24).

The dietary supplement consisted of 2 g L-tryptophan, 10 g L-tyrosine, blueberry juice, and blueberry extract. These specific dosages of L-tryptophan and L-tyrosine have been shown to significantly elevate free tryptophan and free tyrosine in plasma and to be well tolerated, but not to increase total tryptophan and total tyrosine in breast milk (40, 41). Tryptophan, which also may help initiate sleep, was given in the evening of postpartum day 4, and tyrosine was given on the morning of day 5, to avoid competition at the large neutral amino acid transporters for transport through the blood-brain barrier. The 1-mg L-tryptophan capsules (Apo-tryptophan) were obtained from Apotex, and 500-mg L-tyrosine capsules were obtained from Trophic Canada. The blueberry extract powder VitaBlue was obtained from Future Ceuticals and prepared and packaged at the National Sanitation Foundation International's Guelph Food Technology Centre (NSF-GFTC), in sachets containing 1 g of powder. Blueberry juice was obtained from Milne Fruit Products and packaged at the GFTC in 280-mL bottles. At the point of consumption, the blueberry juice and extract were mixed to form a readily digestible, highly palatable beverage. The blueberry extract was added to the blueberry juice because of the anthocyanin damage that occurs during the pasteurization of blueberry juice.

Because this was the first administration of the supplement to postpartum women, an open trial design was chosen. Initially, the first 60% of the subjects were assigned to the control group, followed by recruitment of 60% of the supplement group. Afterward, recruitment was run concurrently, with separate advertisements for each group alternated to obtain continuous enrollment in each group. The study was described as a trial to aid research in the area of PPD. Given that this was the first study of this supplement in the postpartum period, prospective subjects were informed that although we had no evidence of the supplement's efficacy, the doses of L-tryptophan and L-tyrosine that were above dietary intake levels were known not to affect the total concentrations of these components in breast milk.

The protocol involved one visit for screening and one visit for measurement of mood. The first visit was a screening visit done during the last trimester of pregnancy. During this visit, subjects were evaluated with the SCID for DSM-IV, BDI, and the NEO PI-R. The second visit was the active protocol day, occurring on postpartum day 5. Shortly after giving birth, women in the supplemented group received the supplement ingredients with an instruction sheet to take the supplement over the course of 3 d, starting on postpartum day 3 (Table 2). At the time of tryptophan administration, the subjects also received a phone call. Tyrosine and blueberry juice/extract administration (the only challenging part of the protocol, owing to the number of tyrosine capsules) was visually verified on postpartum day 5. On each day of supplement administration, subjects were questioned about the tolerability of the supplements, to record any side effects.

Assessment of Vulnerability to Depressed Mood Induction. Our measurement focused on postpartum day 5 because this is the peak point of depression, mood lability, and PPB severity (2, 23, 51). It is also the time point with the most robust elevation in MAO-A levels throughout the brain, including affect-modulating brain regions, in the early postpartum period (14). PPB is usually negligible before postpartum day 3 and after the peak at day 5 shows a variable rate of decline, but usually resolves by day 10 (23). Timing of the assessments was identical in the control and supplemented groups.

During the second visit, the subjects underwent a neutral MIP, followed by a sad MIP and then another neutral MIP. Measurements of mood were applied before and after the sad MIP. The purpose of the first neutral MIP was to reduce the environmental effects on mood, and the purpose of the second neutral MIP was to remove any effects of the sad MIP (Fig. 1). To induce sad and neutral mood states, the Velten (52) MIP was used in combination with the approach of Clark et al. (53). The Velten method, a series of 60 read self-referent statements, is the most widely used technique for studying affective influences upon behavior and it has demonstrated effectiveness in altering subjective emotional states. Negative statements reflect pessimism, dissatisfaction, or lethargy (e.g., "life is a heavy burden"). An example of a neutral statement is "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, the subjects also listened to music while reading the statements, based on work by Clark et al. (53). The subjects listened to Prokofiev's "Russia Under the Mongolian Yoke" for the sad MIP and to Mozart's "Piano Concerto No. 21 in C Major" for the neutral MIP.

Measures. The primary outcome measure used in this study was the change in the standard VAS (10 cm) for rating depressed mood after the sad MIP. The VAS contains a total of seven items consistent with how the subject feels at the moment. The additional items included ratings for being drowsy, excited, tense, stressed, without energy, and anxious. To represent the baseline VAS, a mean VAS score was calculated from the two VAS scores obtained before the sad MIP (VAS1 and VAS2). To represent the VAS post-MIP, a mean VAS score for the two VAS scores obtained after the sad MIP (VAS3 and VAS4) was calculated (Fig. 1). Two additional VAS scores (VAS5 and VAS6) were obtained afterward as part of a safety protocol to confirm that the subject's depressed mood had been normalized.

For an additional measure of sadness, subjects also completed the POMS (65-adjective version) concomitantly with the VAS. From the POMS, six factors were derived, including depression, tension, anger, fatigue, vigor, and confusion. Measurement of scores attributable to the sad MIP was calculated in the same manner as for the VAS scores.

Statistical Analyses. The primary analysis was a repeated-measures ANOVA with the baseline and post-MIP VAS depressed mood scores as the dependent variables and group (control or supplemented group) as a between-subject factor. The secondary analysis was repeated-measures ANOVA with baseline and post-MIP depression subscale score of the POMS as the dependent variables and group (control or supplemented group) as the between-subject factor.

Regulatory Approvals. This study was approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health in Toronto 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 at any time during the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization's Good Clinical Practice guidelines. This trial is registered at ClinicalTrials.gov (identifier NCT02073175).

ACKNOWLEDGMENTS. This research received project support from the Canadian Institutes of Health Research through the Canada Research Chair in Neurochemistry of Major Depressive Disorder, the Leslois Shaw Foundation, and the Campbell Family Mental Health Research Institute. These organizations did not participate in the design or execution of this study or preparation of the manuscript. Y.D. received studentship salary support from the Ontario Mental Health Foundation.

1. Adewuya AO (2006) Early postpartum mood as a risk factor for postnatal depression in Nigerian women. *Am J Psychiatry* 163(8):1435–1437.
2. O'Hara MW, Schlechte JA, Lewis DA, Wright EJ (1991) Prospective study of postpartum blues: Biologic and psychosocial factors. *Arch Gen Psychiatry* 48(9):801–806.
3. Hannah P, Adams D, Lee A, Glover V, Sandler M (1992) Links between early postpartum mood and post-natal depression. *Br J Psychiatry* 160:777–780.
4. Dennis CL (2004) Can we identify mothers at risk for postpartum depression in the immediate postpartum period using the Edinburgh Postnatal Depression Scale? *J Affect Disord* 78(2):163–169.
5. Harris B, et al. (1994) Maternity blues and major endocrine changes: Cardiff puerperal mood and hormone study II. *BMJ* 308(6934):949–953.
6. O'Hara MW, Swain AM (1996) Rates and risk of postpartum depression: A meta analysis. *Int Rev Psychiatry* 8:37–54.
7. Wisner KL, Parry BL, Piontek CM (2002) Clinical practice: Postpartum depression. *N Engl J Med* 347(3):194–199.
8. Werner E, Miller M, Osborne LM, Kuzava S, Monk C (2015) Preventing postpartum depression: Review and recommendations. *Arch Women Ment Health* 18(1):41–60.
9. Nott PN, Franklin M, Armitage C, Gelder MG (1976) Hormonal changes and mood in the puerperium. *Br J Psychiatry* 128:379–383.
10. Epperson CN, et al. (2006) Preliminary evidence of reduced occipital GABA concentrations in puerperal women: A 1H-MRS study. *Psychopharmacology (Berl)* 186(3):425–433.
11. Frokjaer VG, et al. (2015) Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: A positron emission tomography study. *Biol Psychiatry* 78(8):534–543.
12. Moses-Kolko EL, et al. (2008) Serotonin 1A receptor reductions in postpartum depression: A positron emission tomography study. *Fertil Steril* 89(3):685–692.
13. Sacher J, et al. (2015) Relationship of monoamine oxidase-A distribution volume to postpartum depression and postpartum crying. *Neuropsychopharmacology* 40(2): 429–435.
14. Sacher J, et al. (2010) Elevated brain monoamine oxidase A binding in the early postpartum period. *Arch Gen Psychiatry* 67(5):468–474.
15. Luine VN, McEwen BS (1977) Effect of oestradiol on turnover of type A monoamine oxidase in brain. *J Neurochem* 28(6):1221–1227.
16. Holschneider DP, Kumazawa T, Chen K, Shih JC (1998) Tissue-specific effects of estrogen on monoamine oxidase A and B in the rat. *Life Sci* 63(3):155–160.
17. Gundlah C, Lu NZ, Bethea CL (2002) Ovarian steroid regulation of monoamine oxidase-A and -B mRNAs in the macaque dorsal raphe and hypothalamic nuclei. *Psychopharmacology (Berl)* 160(3):271–282.
18. Smith LJ, Henderson JA, Abell CW, Bethea CL (2004) Effects of ovarian steroids and raloxifene on proteins that synthesize, transport, and degrade serotonin in the raphe region of macaques. *Neuropsychopharmacology* 29(11):2035–2045.
19. Ma ZQ, et al. (1993) Estrogen modulation of catecholamine synthesis and monoamine oxidase A activity in the human neuroblastoma cell line SK-ER3. *J Steroid Biochem Mol Biol* 47(1-6):207–211.
20. Galea LA (2008) Gonadal hormone modulation of neurogenesis in the dentate gyrus of adult male and female rodents. *Brain Res Brain Res Rev* 57(2):332–341.
21. Maguire J, Mody I (2008) GABA(A)R plasticity during pregnancy: Relevance to postpartum depression. *Neuron* 59(2):207–213.
22. Suda S, Segi-Nishida E, Newton SS, Duman RS (2008) A postpartum model in rat: Behavioral and gene expression changes induced by ovarian steroid deprivation. *Biol Psychiatry* 64(4):311–319.
23. Kendell RE, McGuire RJ, Connor Y, Cox JL (1981) Mood changes in the first three weeks after childbirth. *J Affect Disord* 3(4):317–326.
24. Dowlati Y, et al. (2014) Effect of dysfunctional attitudes and postpartum state on vulnerability to depressed mood. *J Affect Disord* 161:16–20.
25. Segal ZV, et al. (2006) Cognitive reactivity to sad mood provocation and the prediction of depressive relapse. *Arch Gen Psychiatry* 63(7):749–755.
26. Meyer JH, et al. (2006) Elevated monoamine oxidase a levels in the brain: An explanation for the monoamine imbalance of major depression. *Arch Gen Psychiatry* 63(11):1209–1216.
27. Meyer JH, et al. (2009) Brain monoamine oxidase A binding in major depressive disorder: Relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence. *Arch Gen Psychiatry* 66(12):1304–1312.
28. Rekkas PV, et al. (2014) Greater monoamine oxidase A binding in perimenopausal age as measured with carbon 11-labeled harmine positron emission tomography. *JAMA Psychiatry* 71(8):873–879.
29. Johnson S, et al. (2011) The reduction of R1, a novel repressor protein for monoamine oxidase A, in major depressive disorder. *Neuropsychopharmacology* 36(10): 2139–2148.
30. Saura J, Kettler R, Da Prada M, Richards JG (1992) Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 in vitro: Localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. *J Neurosci* 12(5): 1977–1999.
31. Tong J, et al. (2013) Distribution of monoamine oxidase proteins in human brain: Implications for brain imaging studies. *J Cereb Blood Flow Metab* 33(6):863–871.
32. Youdim MB, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci* 7(4):295–309.
33. Bouayed J, Rammal H, Soulimani R (2009) Oxidative stress and anxiety: Relationship and cellular pathways. *Oxid Med Cell Longev* 2(2):63–67.
34. Brown NC, Andreazza AC, Young LT (2014) An updated meta-analysis of oxidative stress markers in bipolar disorder. *Psychiatry Res* 218(1-2):61–68.
35. Andres-Lacueva C, et al. (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr Neurosci* 8(2):111–120.
36. Bel N, Artigas F (1996) Reduction of serotonergic function in rat brain by tryptophan depletion: Effects in control and fluvoxamine-treated rats. *J Neurochem* 67(2): 669–676.
37. Kalt W, et al. (2008) Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. *J Agric Food Chem* 56(3):705–712.

38. McTavish SF, Cowen PJ, Sharp T (1999) Effect of a tyrosine-free amino acid mixture on regional brain catecholamine synthesis and release. *Psychopharmacology (Berl)* 141(2):182–188.
39. Bongiovanni R, Newbould E, Jaskiw GE (2008) Tyrosine depletion lowers dopamine synthesis and desipramine-induced prefrontal cortex catecholamine levels. *Brain Res* 1190:39–48.
40. Dowlati Y, et al. (2014) No effect of oral tyrosine on total tyrosine levels in breast milk: Implications for dietary supplementation in early postpartum. *Arch Women Ment Health* 17(6):541–548.
41. Dowlati Y, et al. (2015) No effect of oral L-tryptophan or alpha-lactalbumin on total tryptophan levels in breast milk. *Eur Neuropsychopharmacol* 25(6):779–787.
42. Leyton M, et al. (1997) The effect of tryptophan depletion on mood in medication-free, former patients with major affective disorder. *Neuropsychopharmacology* 16(4):294–297.
43. Shurtleff D, Thomas JR, Schrot J, Kowalski K, Harford R (1994) Tyrosine reverses a cold-induced working memory deficit in humans. *Pharmacol Biochem Behav* 47(4):935–941.
44. Leyton M, et al. (2004) Decreasing amphetamine-induced dopamine release by acute phenylalanine/tyrosine depletion: A PET/[11C]raclopride study in healthy men. *Neuropsychopharmacology* 29(2):427–432.
45. Chabrol H, Coroner N, Rusibane S, Séjourné N (2007) [A pilot study of prevention of postpartum blues]. *Gynecol Obstet Fertil* 35(12):1242–1244. French.
46. Doornbos B, et al. (2009) Supplementation of a low dose of DHA or DHA+AA does not prevent peripartum depressive symptoms in a small population based sample. *Prog Neuropsychopharmacol Biol Psychiatry* 33(1):49–52.
47. Dennis CL, Dowswell T (2013) Psychosocial and psychological interventions for preventing postpartum depression. *Cochrane Database Syst Rev* 2(2):CD001134.
48. Fernstrom JD (2012) Effects and side effects associated with the non-nutritional use of tryptophan by humans. *J Nutr* 142(12):2236S–2244S.
49. O'Brien C, Mahoney C, Tharion WJ, Sils IV, Castellani JW (2007) Dietary tyrosine benefits cognitive and psychomotor performance during body cooling. *Physiol Behav* 90(2-3):301–307.
50. First M, Spitzer R, Williams J, Gibbon M (1995) *Structured Clinical Interview for DSM-IV, Non-Patient Edition (SCID-NP, Version 1.0)* (American Psychiatric Press, Washington, DC).
51. O'Hara MW, Schlechte JA, Lewis DA, Varner MW (1991) Controlled prospective study of postpartum mood disorders: psychological, environmental, and hormonal variables. *J Abnorm Psychol* 100(1):63–73.
52. Velten E, Jr (1968) A laboratory task for induction of mood states. *Behav Res Ther* 6(4):473–482.
53. Clark DM (1985) The Velten Mood Induction Procedure and cognitive models of depression: A reply to Riskind and Rholes (1985). *Behav Res Ther* 23(6):667–669.