The Relationship Between Maternal Tryptophan Metabolism, Cytokines And Cortisol In Term And Preterm Expressed Breast Milk

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Table of Contents

Abstract........................................................................................................................................... i
Key Words and Acknowledgements ............................................................................................... i
Introduction ........................................................................................................................................ 1
Methods ........................................................................................................................................... 4
Results ............................................................................................................................................... 6
Discussion ........................................................................................................................................ 12
Limitations ....................................................................................................................................... 13
Implications and Recommendations ............................................................................................... 14
References ....................................................................................................................................... 16

List of Figures

Figure 1. Tryptophan Metabolism Pathways.................................................................................. 2
Figure 2. Tryptophan levels in Term v Preterm EBM from day 7 to day 14. ... 7
Figure 3. Kynurenine levels in Term v Preterm (PT) EBM on Day 7 & 14 ...... 8
Figure 4. Kynurenic Acid levels in Term & Preterm EBM on Day 7 & 14........ 8
Figure 5. Kynurenine to Tryp Ratio in Term & Preterm EBM Day 7 & 14...... 9
Figure 6. TNF-α levels in Preterm EBM on Day 7 & 14................................. 10
Figure 7. IL-8 levels in Preterm EBM on Day 7 & 14........................................ 10
Figure 8. Cortisol levels in Term and Preterm (PT) EBM on Day 7 & 14...... 11

List of Tables

Table 1. Characteristics of Mothers and Infants........................................................................... 7
Table 2. Pro-inflammatory Cytokine Levels in Term & Preterm EBM on Day 7 & Day 14. ......................... 11
Abstract

Motivations/ Problem Statement
Tryptophan (TP), present in human breast milk, is an essential amino acid and a precursor for the key neurotransmitter serotonin. TP is vital for optimal growth in early infancy and for the optimal development of the brain. The relationship of TP to its neuroactive pathway metabolites kynurenine (Kyn) and kynurenic acid (KyA), immunity and maternal stress in preterm and term expressed breast milk (EBM) has not previously been investigated. This study will investigate the relationship of serotonin precursor TP and its neuroactive pathway metabolites Kyn and KyA, in preterm and term EBM in the first 14 days following birth, and the relationship of TP to maternal stress (EBM cortisol) and immune status (EBM cytokines - IFN-γ, TNF-α, IL-1, 6, 8), key regulators of TP metabolism.

Methods/ Approach
24 mothers were recruited from a Maternity Hospital: 12 term (> 38 weeks) and 12 preterm (< 35 weeks). EBM samples were collected on day 7 and 14. TP, Kyn and KyA were measured using HPLC, EBM cytokines using the MSD assay system, and EBM Cortisol using a Cortisol ELISA kit.

Results/ Findings
Over the duration of the study, TP levels were significantly lower in preterm EBM by comparison to term EBM (p< 0.05). Kyn and KyA levels, and the Kyn to TP ratio increased significantly in term EBM from day 7 to day 14 (p< 0.01). An effect of time only was identified for EBM TNF-α and IL-8 concentrations (p< 0.05). There were no significant changes in EBM cortisol.

Conclusions/ Implications
While this is a small study, if the low levels of TP and its metabolites in preterm EBM are reflected in future larger studies and if the low levels are replicated in infant plasma circulating levels, this may denote significant implications for the neurological development of vulnerable exclusively breastfed preterm infants.
Key words:

Human milk, Tryptophan, Kynurenine, Kynurenic acid, Cortisol

Acknowledgements

A special thanks to all the mothers who so enthusiastically participated in the study.
Introduction

Breast milk is an excellent source of nutrition for growing infants due to its numerous bioactive factors such as cytokines, growth factors and hormones (Castellote et al. 2011). Newborns, with immature immune systems, are initially limited in their ability to respond to infection (Garofalo 2010). Breast milk, abundant in immunoregulatory cytokines and chemokines, continues the infants’ exposure to the mother’s immune system, garnering passive immunity (Agarwal et al. 2011) and playing a primary role in nurturing and regulating the maturation of the immune system (Castellote et al. 2011, Garofalo 2010). For exclusively breast-fed infants, breast milk is the only source of the neuroactive compound tryptophan (TP).

TP is an essential amino acid that can only be acquired through diet in humans (Flores-Cruz and Escobar 2012). It is a major source for protein synthesis in all cells in the body (Ruddick et al. 2006), and is needed for the regulation of immune responses, behavior, mood and growth (Floc'h et al. 2011). TP is a precursor for hormones serotonin and melatonin. 1% of dietary TP is utilised for serotonin and melatonin synthesis while 99% is metabolized via the Kyn pathway (Luigi et al. 2013). Two enzymes, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), are involved in the Kyn pathway of TP metabolism. TDO, mainly expressed in the liver, is a stress responsive enzyme and is also induced when there is an excess of TP in plasma and tissues (Floc'h et al. 2011). IDO is an immune responsive enzyme and is expressed in numerous tissues such as the intestines, lungs, the placenta (Guanyou et al. 2012), immune cells, astrocytes and dendritic cells in the central nervous system (Ruddick et al. 2006, Schröcksnadel et al. 2006). Both the TDO and IDO pathways lead to the production of metabolites Kyn, neuroprotective kynurenic acid (KyA), and neurotoxic 3-hydroxykynurenine and quinolinic acid (Schwarcz et al. 2012). See Figure 1 for tryptophan metabolism pathways.
Figure 1. Tryptophan Metabolism Pathways

Maternal serum levels of TP fluctuate quite considerably during pregnancy with the availability of TDO and IDO being tightly regulated both during and after pregnancy (Schröcksnadel et al. 2006). It has been established that IDO is necessary for the maintenance of pregnancy as it protects the foetus from T-cell driven local inflammatory responses (Mellor et al. 2001), thus inducing immune tolerance to the fetus (Schröcksnadel et al. 2003). The Schröcksnadel et al. (2003) landmark study demonstrated: Serum TP levels progressively decrease during pregnancy with the lowest TP concentrations present at term, accompanied by increases in immune activation marker neopterin (suggesting immune activated IDO breakdown of TP) and increases in Kyn, and the Kyn to TP ratio (a marker of TP breakdown); in the postpartum period, while TP began to rise again, Kyn and the Kyn to TP ratio also remained elevated, despite the normalisation of immune activation markers, accompanied also by an elevation in liver enzyme ALT. This suggests that immune activated IDO breakdown of TP ceases in the immediate postpartum period and may be replaced by stress responsive hepatic TDO breakdown of TP (though at a smaller scale). It is further
suggested that critically low TP depletion in the immediate postpartum period is contributory to maternal postpartum mood disorders and even psychosis (Anderson and Maes 2013, Maes et al. 2011).

Cortisol, present in breast milk, is a stress-sensitive steroid hormone (glucocorticoid) that is released under the control of the hypothalamic-pituitary-adrenal (HPA) axis, and is vital for the correct development of the central nervous system (Glynn et al. 2007). Cortisol is the main hormone mediator of stress (Harris and Seckl 2011). During the prenatal period, while glucocorticoids are vital for normal development of the foetus (Harris and Seckl 2011), excess glucocorticoids due to excess maternal stress are strongly associated with pre-term birth (Silva et al. 2009, Roy-Matton et al. 2011, Baibazarova et al. 2013). It has also been shown that excess maternal stress in the prenatal period is associated with behaviour, mood, cognition and attention abnormalities in later life (Gutteling et al. 2005, Gutteling et al. 2006). In the postnatal period, higher levels of maternal cortisol are associated with fearfullness in breastfed infants (Glynn et al. 2007). Cortisol activates TDO led breakdown of TP, leading directly to a decrease in brain serotonin synthesis (Floc'h et al. 2011). This disturbance in serotonergic function is also seen as a significant causative factor to the development of anxiety, aggression, affective disorders and stress syndromes (Floc'h et al. 2011). Further, melatonin, the by-product of serotonin, also found in breast-milk, is crucially important in the regulation of numerous physiological responses (Ruddick et al. 2006). It plays a major role in stabilising the circadian rhythm of the newborn (Cubero et al. 2005), for optimal development of the brain (Aparicio et al. 2007),and in immune defence (Honorio-França et al. 2013). Therefore, any alterations in TP supply early in life can have major neurodevelopmental consequences (Cubero et al. 2005).

The relationship dynamic between serotonin precursor TP, its neuroactive pathway metabolites (Kyn and KyA), immunity and stress has not to the author’s knowledge been previously investigated in term and preterm expressed breast milk (EBM). In this study, it was hypothesized that the higher stress levels associated with pre-term birth would be reflected in
preterm EBM, resulting in increased levels of cortisol and decreased levels of TP by comparison to term EBM. The aims of the study are twofold: (a) Investigate and compare the relationship of serotonin precursor TP and its neuroactive pathway metabolites Kyn and KyA, in preterm and term EBM in the first 14 days following birth, and (b) examine the relationship of TP to maternal stress (EBM cortisol) and immune status (EBM cytokines - IFN-γ, TNF-α, IL-1, IL-6, IL-8) over the same time-frame.

**Methods**

This study was conducted in the neonatology department of a maternity hospital over an eight-week period in June and July 2013. All mothers received verbal and written information about the study and signed a written consent form prior to participation. General medical data was collected on each mother and baby - apgar scores at 1 and 5 minutes, birth weight and gestation, vaginal or caesarian delivery, mother’s age, parity, allergies, occupation etc.

Two specimens of EBM were collected from all mothers post-partum on day 7 and on day 14 from their homes. All specimens were collected in the morning by manual expression into sterile polypropylene containers at the end of infant feeding (hind-milk). All EBM samples were stored initially at -20°C and then at -80°C until assayed. The exclusion criteria included maternal use of antibiotics, maternal mastitis, and mothers living further than a thirty miles radius from the hospital. Five mothers who were initially recruited onto the study had to be excluded (four mothers were commenced on antibiotics and one mother had difficulties with breastfeeding). The sample size of this exploratory study was based on previous studies in IBS which demonstrated that 10 subjects was sufficient to detect differences in TP metabolism between IBS and healthy controls (Clarke et al. 2009). The study was granted ethics approval in May 2013.
All EBM sample analysis was conducted in a university laboratory. TP and its Kyn pathway metabolites were determined using high performance liquid chromatography (HPLC) coupled to fluorescent and UV detection as previously described (Clarke et al. 2009). Briefly, EBM samples were spiked with internal standard (3-Nitro L-tyrosine) prior to the addition of 20 µl of 4M perchloric acid to 200 µl of sample. Samples were centrifuged at 21000g on a Hettich Mikro 22R centrifuge (AGB, Dublin, Ireland) for 20 minutes at 4°C and 100 µl of supernatant transferred to a HPLC vial for analysis on the HPLC system (UV and FLD detection). All samples were injected onto a reversed phase Luna 3 µm C18 (2) 150 × 2 mm column (Phenomenex), which was protected by Krudkatcher disposable pre-column filters (Phenomenex) and Security Guard cartridges (Phenomenex). The mobile phase consisted of 50 mM acetic acid, 100 mM zinc acetate with 3% (v/v) acetonitrile and was filtered through Millipore 0.45 µm HV Durapore membrane filters (AGB) and vacuum degassed prior to use. Compounds were eluted isocratically over a 30-minute runtime at a flow rate of 0.3 mls/min after a 20 µl injection. The column was maintained at a temperature of 30°C and samples/standards were kept at 8°C in the cooled autoinjector prior to injection. The fluorescent detector was set at an excitation wavelength of 254 nm and an emission wavelength of 404 nm. The UV detector was set to 330 nm. TP and its metabolites (Kyn, KyA) were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Analyte:Internal standard peak height ratios were measured and compared with standard injections and results were expressed as ng of analyte per ml of EBM.

A Cortisol ELISA kit (Enzo Life Sciences) was used to determine the concentration of cortisol in EBM. Samples were thawed on ice and centrifuged at 2000 x g for 10 min @ 4°C to de-fat the EBM. The aqueous phase was extracted and used in the 96-well plate enzyme-linked immunosorbent assay. Analysis was carried out as per manufacturer’s instructions. Optical density was read immediately at 405 nm. All samples were run in duplicate and their concentration determined using a standard curve. Results are expressed as picograms per millilitre of sample. For cytokine analysis, a Meso scale
Discovery (MSD) assay system was used. The EBM was prepared as described for cortisol assays. Samples were centrifuged at 2000 x g for 10 min @ 4°C and the aqueous layer aspirated. A Human Pro-inflammatory Il 4-Plex Ultra-Sensitive Kit (MSD) was used to determine IL-1β, IL-6, IL-8 and TNF-α concentration in the EBM samples. A Single Spot Human Cytokine Assay System (MSD) for IFN-γ was also run. Samples and standards were run in duplicate according to the manufacturer’s instructions and the concentration was measured using the MSD Discovery Workbench® analysis software. Results are presented in picograms per millilitre of sample.

Statistical analysis was performed using the Graphpad Prism software package. Data were analysed using Two way ANOVA repeated measures (matched pairs only) and Two way ANOVA (all data points, mission values not replaced) or post-hoc analysis (Bonferroni multiple comparisons) as appropriate.

**Results**

The demographics of the mothers and infants are presented in Table 1. A total of 29 lactating mothers were recruited, of whom 24 successfully completed the study: 12 mothers with term babies (>38 weeks, group T), and 12 mothers with pre-term babies (< 35 weeks, group PT).
Table 1. Characteristics of Mothers and Infants.
Mean ± SD, and range

<table>
<thead>
<tr>
<th></th>
<th>Group T, n=12</th>
<th>Group PT, n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age, year</td>
<td>34.4 ± 3.75 (29-44)</td>
<td>37 ± 7.16 (29-44)</td>
</tr>
<tr>
<td>Parity</td>
<td>1.92 ± 0.9 (1-3)</td>
<td>1.71 ± (1-4)</td>
</tr>
<tr>
<td>Parturition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%SVD / %LSCS</td>
<td>67/33</td>
<td>25/75</td>
</tr>
<tr>
<td>Gestational Age, week</td>
<td>39.7 ± 0.9 (38.1-41.7)</td>
<td>32.6 ± 1.8 (29-34.4)</td>
</tr>
<tr>
<td>Birth Weight, kg</td>
<td>3.5 ± 0.6 (2.6-4.3)</td>
<td>2.0 ± 0.4 (1.4-2.8)</td>
</tr>
</tbody>
</table>

Over the duration of the study, TP levels were significantly lower overall in preterm EBM by comparison to term EBM (p<0.05, all data points, see Figure 2). There was no effect of time on TP concentrations (p > 0.05), nor was there an interaction between time and group. A post hoc analysis did not indicate any differences between groups (p > 0.05). A matched pair analysis indicated no significant effect of group, time, or interaction between group and time (p > 0.05).

Figure 2. Tryptophan levels in Term versus Preterm EBM from day 7 to day 14 (Results are expressed as the mean ± SEM).
Analysis of Kyn levels in term EBM indicated an effect of time (p<0.001) but no effect of group and no interaction between group and time (p > 0.05, all data points). A matched pair analysis of Kyn levels in term EBM also highlighted an effect of time (p<0.001). A post hoc analysis indicated that Kyn concentrations increased significantly in term EBM from day 7 to day 14 (22.1 ± 3.7 V 72.5 ± 8.4 ng/ml, p<0.01, see Figure 3). The Kyn level increase was not significant in preterm EBM from day 7 to day 14 (23.6 ± 5.7 V 46.9 ± 10.5 ng/ml, p > 0.05).

![Figure 3. Kynurenine levels in Term and Preterm (PT) EBM on Day 7 and Day 14 (Results are expressed as the mean ± SEM).](image)

Analysis of neuroprotective KyA levels in term EBM indicated an effect of time (p<0.001) but no effect of group and no interaction between group and time (p > 0.05, all data points). A matched pair analysis of KyA levels in term EBM also highlighted an effect of time (p<0.001). A post hoc analysis indicated KyA levels increased significantly in term EBM from day 7 to day 14 (10.8 ± 1.8 V 41.7 ± 10.3 ng/ml, p<0.01, see Figure 4). The KyA level increase was not significant in preterm EBM from day 7 to day 14 (13.6 ± 2.6 V 21.5 ± 4.7 ng/ml, p > 0.05).
Figure 4. Kynurenic Acid levels in Term and Preterm (PT) EBM on Day 7 and Day 14 (Results are expressed as the mean ± SEM).

Analysis of the Kyn to TP ratio, an indicator of TP breakdown, demonstrated an effect of time ($p<0.01$, all data points) but no effect of group and no interaction between group and time. A matched pair analysis of the Kyn to TP ratio in term EBM indicated a trend towards an effect of time ($p=0.05$). The Kyn to TP ratio increased significantly from day 7 to day 14 in term EBM (0.029 ± 0.003 V 0.11 ± 0.02 ng/ml, $p<0.01$, see Figure 5). The Kyn to TP ratio increase was not significant in preterm EBM (0.06 ± 0.02 V 0.08 ± 0.01 ng/ml, $p>0.05$).
The analysis indicated an effect of time only for TNF-α and IL-8 (p < 0.05) with no effect on group (p > 0.05) and no interaction effect (p > 0.05), see Figure 6 and 7. There was no effect of group or time and no interaction between group and time for other EBM pro-inflammatory cytokines (IFN-γ, IL-1, IL-6), see Table 2.
Figure 7. IL-8 levels in Preterm (PT) EBM on Day 7 and Day 14 (Results are expressed as the mean ± SEM).

Table 2. Pro-inflammatory Cytokine Levels in Term (T) and Preterm (PT) EBM on Day 7 & Day 14, and p-Values for Group (G), Time (Ti) and Interaction (I).

Results (pg/ml) are expressed as the mean ± SEM.

<table>
<thead>
<tr>
<th>T/PT</th>
<th>Day 7</th>
<th>Day 14</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>T: 0.9 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.1 (G)</td>
</tr>
<tr>
<td></td>
<td>PT: 0.3 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.3 (Ti)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 (I)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>T: 17.2 ± 4.1</td>
<td>9 ± 2.3</td>
<td>0.9 (G)</td>
</tr>
<tr>
<td></td>
<td>PT: 13.6 ± 3.8</td>
<td>10.6 ± 3.1</td>
<td>0.03 (Ti)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.7 (I)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>T: 18.4 ± 6.1</td>
<td>11.4 ± 4.7</td>
<td>0.6 (G)</td>
</tr>
<tr>
<td></td>
<td>PT: 16.1 ± 8.4</td>
<td>9.3 ± 2.6</td>
<td>0.2 (Ti)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 (I)</td>
</tr>
<tr>
<td>IL-6</td>
<td>T: 22.2 ± 5.9</td>
<td>9.1 ± 2.9</td>
<td>0.9 (G)</td>
</tr>
<tr>
<td></td>
<td>PT: 20.6 ± 7.6</td>
<td>9.4 ± 3.5</td>
<td>0.06 (Ti)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.6 (I)</td>
</tr>
<tr>
<td>IL-8</td>
<td>T: 440.2 ± 112.7</td>
<td>267.9 ± 74.8</td>
<td>0.6 (G)</td>
</tr>
<tr>
<td></td>
<td>PT: 384.2 ± 148.3</td>
<td>232.7 ± 73.3</td>
<td>0.03 (Ti)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0 (I)</td>
</tr>
</tbody>
</table>

There was no effect of group or time and no interaction between group and time for EBM cortisol, see Figure 8.

Figure 8. Cortisol levels in Term and Preterm (PT) EBM on Day 7 and Day 14 (Results are expressed as the mean ± SEM).
Discussion

In this study, the supply dynamics of TP and its Kyn pathway metabolites (Kyn and KyA), cortisol and cytokines, in paired EBM samples from mothers of term and preterm infants were investigated. To the author’s knowledge, this is the first demonstration showing that preterm EBM contains less TP than term EBM. In addition, TP metabolites, Kyn and KyA, have been identified and quantified in EBM. Notably, higher levels of these metabolites were found in term day 14 EBM by comparison to term day 7 EBM.

Contrary to the original hypothesis that the higher stress levels associated with preterm birth would be reflected in preterm EBM, the mothers of preterm infants did not exhibit elevated levels of stress as indicated by EBM cortisol concentrations. As cortisol triggers TDO-led breakdown of TP down the Kyn metabolite pathway and because our results showed less TP in preterm EBM, it would have been reasonable to speculate that there might be higher levels of cortisol in preterm EBM. Equally, as cytokines activate IDO-led breakdown of TP down the Kyn metabolite pathway, one may also have expected to find higher levels of immune activation in preterm EBM. Again, this was not the case. Although no group differences in cytokine output over time were observed, the overall decrease in TNF-α and IL-8 from day 7 to day 14 runs contrary to the hypothesis. Consequently, it remains unclear why the concentration of TP and its metabolites differ between term and preterm EBM. It should be noted, however, that using EBM cortisol measures as a proxy for more validated sources (e.g. plasma or saliva) may not be totally ideal and requires further investigation. Breast milk is widely recognized as being notoriously difficult to examine and quantify due to its “biochemical complexity”, the small concentration of many of its bioactive components, and the fact that it is subject to many dynamic and qualitative changes during lactation (Garofalo 2010), including exhibiting circadian rhythms (Andrade Silva et al. 2013, Honorio-França et al. 2013). It should also be noted that the small number of EBM samples taken at only one time-point each day in this
exploratory study may have impaired the ability to reliably and accurately detect the group differences of these bioactive components.

The core findings however, if replicated, could have significant implications. Breast milk is the only source of the essential amino acid TP for exclusively breast-fed infants. TP is vital for optimal growth (protein anabolism) in early infancy (Sánehez et al. 2013), and for the optimal development of the CNS (Cubero et al. 2005). At no other time is structural demand for TP higher than in the neonatal period (Sánehez et al. 2013). Serotonin is needed for the regulation of the GI system, appetite, mood, growth and haemodynamics (Floc'h et al. 2011). Brain serotonin synthesis is proportional to and dependent on the level and transport of TP into the brain, thus any TP deficiency in peripheral availability has a direct negative effect on brain serotonin synthesis (Floc'h et al. 2011, Flores-Cruz and Escobar 2012). Decreased serotonin in adults increases the risk of developing mood disturbance, depression and cognitive impairment (Schröcksnadel et al. 2006). Serotonin is a precursor for melatonin. Melatonin, present in breast milk, is vital for immune defense (Honorio-França et al. 2013), for optimal development of the brain (Aparicio et al. 2007), and for consolidation of the sleep-wake cycle (Cubero et al. 2005) in the infant. The above data poses a critical question: If the low levels of TP and its metabolism in preterm EBM are reflected in infant plasma, what effect does this have on the neurological development of vulnerable exclusively breastfed preterm infants?

Limitations

It was not possible to have an exact measure of how much breast milk the infants in this study consumed. This is relevant in that it is possible that TP deficiencies found in preterm EBM in our study could be compensated for by infants who consumed more EBM than their term counterparts. EBM might not be the best biological source to use as a predictive weathervane when looking at maternal stress and immune status. Combining EBM analysis with maternal and infant serum, saliva samples, and validated rating scales to capture the self-reported stress status of the mothers would provide a more
complete picture and should be incorporated into future studies arising from this work. Maternal plasma TP levels were not measured. Consequently, it was not possible to determine if the TP and metabolite concentrations present in EBM are a consequence of enzymatic activity in the breast itself or are reflective of circulating maternal serum concentration. Similarly, because access to infant plasma samples was not possible, it could not be determined if altered TP and Kyn metabolite supply translated into altered circulating availability in the infant. Once these valuable, difficult to acquire samples are harvested, it will also be important to establish the extent to which the free TP concentration in EBM reported here contributes to circulating levels relative to the proportion derived from breast milk proteins (Csapo and Salaman 2009).

Implications and Recommendations

If the lower levels of TP in preterm EBM are replicated and validated in larger studies, future research would be needed to establish the merits of matching full term complement of TP and its metabolites to preterm EBM. Importantly, this could help inform optimal formula milk composition. In breast milk, TP exhibits a dynamic circadian rhythm with higher levels at night, while in formula this is unmatched with static TP concentrations (Sánehez et al. 2013). Of note, Aparicio et al. (2007) state that the average TP concentration in breast milk is 2.5% while in many milk formulas, the average is 1-1.5%.

An intricate and complex balance exists between TP and its Kyn pathway metabolites (Ruddick et al. 2006, Schwarcz et al. 2012)). Peripheral TP and Kyn readily cross the blood-brain-barrier (BBB) via competitive large amino acid transporters, while KyA, 3-hydroxykynurenine and quinolinic acid cross the BBB at relatively low rates (Raison et al. 2010). A high ratio of KyA to 3-hydroxykynurenine and quinolinic acid is associated with low cognitive performance (e.g. schizophrenia), while a low ratio of KyA to 3-hydroxykynurenine and quinolinic acid is associated with high neuronal vulnerability (e.g. Huntingtons disease) (Schwarcz et al. 2012). Future research should set out to identify the EBM metabolite profile downstream of Kyn production along the neurotoxic arm of the pathway. Clearly, if neurotoxic
metabolites such as 3-hydroxykynurenine and quinolinic acid are also present in breast milk in addition to KyA, the relevance for the vulnerable infant CNS needs to be established.

With regard to cytokines, research with larger sample sizes is required. While it has been established that cytokines in breast milk confer passive immunity to the infant, they also play an integral role in nurturing and regulating the maturation of the immune system of the infant (Garofalo 2010, Castellote et al. 2011). Further research would be valuable in examining the hypothesis that TP and its metabolites present in breast milk could play a comparable nurturing and regulating function on the immature TP and Kyn pathways in the infant.
References


Maes, M., Leonard, B. E., Myint, A. M., Kubera, M. and Verkerk, R. (2011) 'The new '5-HT' hypothesis of depression: Cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental


