2013-04-10

The role of micronutrients in maternal mood and child neurodevelopment

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The role of micronutrients in maternal mood and child neurodevelopment

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF COMMUNITY HEALTH SCIENCES

CALGARY, ALBERTA

APRIL, 2013

Abstract

A key factor in the prevention and treatment of perinatal depression may be to improve overall micronutrient intake in pregnant women, which also appears to be fundamental to child cognitive and behavioural development. From the global literature, seven conceptual models helped to interpret the mechanism(s) by which micronutrients might influence mental health function. Further review of the literature revealed credible links between nutrient deficiency and mood for folate, vitamin B12, calcium, iron, selenium, zinc and omega-3 fatty acids. In the analysis of a longitudinal pregnancy cohort called the Alberta Pregnancy Outcomes and Nutrition study, women with lower depression scores had higher mean intakes of selenium ($p = 0.0015$) and omega-3s ($p = 0.01$), while women with higher depression scores were less likely to have been born in Canada ($p = 0.01$), more likely to have greater number of chronic conditions ($p = 0.05$) and stressful life events during pregnancy ($p = 0.02$), and lower prenatal and postnatal support ($p = 0.0043$ and $p = 0.0001$, respectively). Adjusting for covariates and nutrients associated with postpartum depression, women with high prenatal depression scores had increased odds of postpartum depressive symptoms (second and third trimester OR = 3.29, 95% CI = 1.55 - 7.01, $p = 0.004$ and OR = 4.26, 95 CI = 2.05 - 8.85, $p < 0.0001$, respectively), while prenatal supplemental selenium (per 10 µg, OR = 0.76, 95% CI = 0.74 - 0.78, $p = 0.0019$) and postnatal social support (OR = 0.87, 95% CI = 0.78 - 0.97, $p = 0.0015$) were protective. For child neurodevelopment, micronutrients are crucial to neuronal function and synaptic plasticity, and ultimately, brain health and mental function. A systematic review found perinatal intake of multi-micronutrients and n-3 fatty acids had some positive effect on child neurodevelopment, but the results were inconclusive, and more research was needed. Overall findings indicated multi-

micronutrients may confer benefits to mental health in pregnant women, and to child behavioural and neurocognitive function.

Preface

This manuscript-based thesis contains four published articles. Manuscript one was a collaborative effort by both authors. For manuscripts two to four, the first author, Brenda Leung, undertook the literature review, conducted the analyses, interpreted the results, and wrote the manuscripts. The manuscripts were developed with the guidance of the senior author (and supervisor), Dr. Bonnie Kaplan. All co-authors provided critical reviews of the manuscripts and contributed intellectual content. These articles have been reproduced in their entirety and included as chapters within this thesis; permission was sought from the respective publishers, and granted in cases (e.g. not open access) where applicable.

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Acknowledgements

This has been a long and arduous journey, and one does not take a journey such as this alone. I want to extend my gratitude to the many people who has helped me along the way.

A special thank you to my supervisor, Bonnie, for her mentorship and friendship, for all her support and encouragement, especially during those times when I was ready to call it quits.

I thank my mom, Li-Yen, for setting me onto this path to being ferociously independent.

A big thank you to my sister, Janet, for being a sounding board and keeping me grounded.

To my nephews Shyam and Eshin, my niece Ari, for their antics that have made me smile. *It is through the eyes of a child do I see that the more I learn, the less I know for sure*.

Finally I want to acknowledge all the women who took part in the APrON study; thank you for sharing your experiences, and giving of your time and energy to this project.

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List of Symbols, Abbreviations and Nomenclature

Abbreviation	Definition
25(OH)D	25-hydroxyvitamin D
AA	Arachidonic acid
AD	Antenatal depression
ADHD	Attention deficit hyperactivity disorder
AI	Adequate Intake
ALSPAC	Avon Longitudinal Study of Parents and Children
APrON	Alberta Pregnancy Outcomes and Nutrition (study)
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BPRS	Brief Psychiatric Rating Scale
CES-D	Center for Epidemiologic Studies Depression
CGI-S and CGI-I	Clinical Global Impressions Severity & Improvement Scales
CIDI	Composite International Diagnostic Interview
DALYs	Disability adjusted lives per years
DHA	Docosahexaenoic acid
DNBC	Danish National Birth Cohort
DRI	Dietary Reference Intakes
DSM	Diagnostic and Statistical Manual of the American Psychiatric Association
EAR	Estimated Average Requirement

Abbreviation	Definition
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
EPDS	Edinburgh Postpartum Depression Scale
FFQ	Food Frequency Questionnaire
HAM-D	Hamilton Rating Scale for Depression
MADRS	Montgomery-Asberg Depression Rating Scale
NHANES	National Health and Nutrition Examination Survey
OQ	Outcome Questionnaire
PD	perinatal depression
POMS-BI	Profile of Moods States - Bipolar Form
PPD	Postpartum depression
PUFA	Polyunsaturated fatty acid
RCT	Randomized controlled trial
RDA	Recommended Dietary Allowance
SF-36	Medical Outcome Study Short Form 36
SIQ	Supplement Intake Questionnaire
UL	Tolerable Upper Intake Level
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Nutrients such as vitamins, minerals, and fatty acids are essential foundations to human health. The impact of nutrition on physical health has been the focus of much research to date. For example, studies have shown how poor nutrition is associated with diseases such as cancer, cardiovascular disease, osteoporosis, and diabetes (1-3). It is only recently that studies are demonstrating an association between nutrient inadequacies and mental health (4, 5). The global rise of mental health problems is a growing problem. Depression is becoming one of the top diseases of burden regardless of socioeconomic levels (6), and women are more likely to be affected than men (7). While malnutrition and nutrient deficiencies are known to be problematic in developing countries, many people may find it surprising that nutrient inadequacies and deficiencies are also quite common in developed nations like Canada (8-10). The impact of poor diet is further magnified by pregnancy, when a woman's nutrient needs increase and the foetus depletes maternal reserves. In fact, the risk for depression during and after pregnancy is a matter of concern in maternal and child health (11). Given the serious consequence of maternal depression (discussed below), and the role of nutrition as a possible risk factor for depression, further study into the association between nutrition and maternal depression is warranted.

The *purpose* of this thesis is examine the role of micronutrients in 1) maternal mental health, specifically perinatal depression, and 2) cognitive and behavioural development of the offspring. To address the knowledge gap on this topic, this thesis will present findings from the international literature and from the Alberta Pregnancy Outcomes and Nutrition (APrON) study.

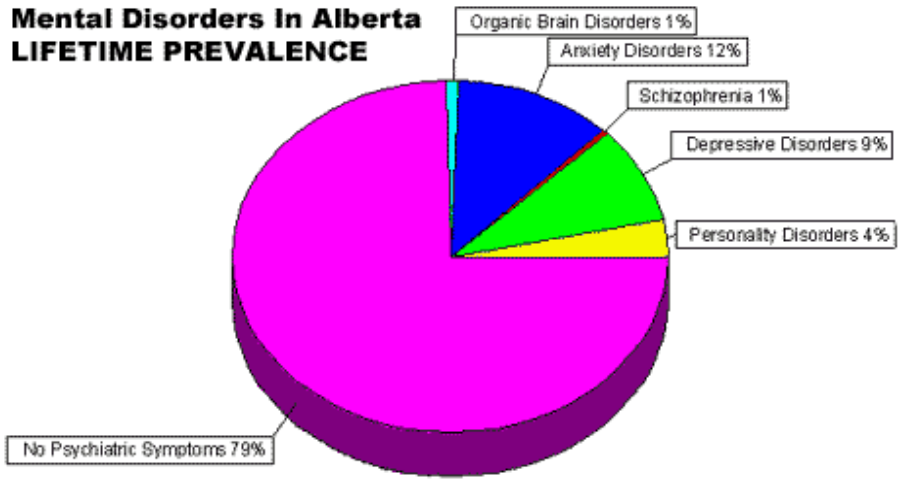
A Review of Depression

Depression is a leading cause of disability worldwide, and one of the top five leading contributors to the global burden of disease according to the World Health Organization (6).

Symptoms of depression are low mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration (12). These problems can be chronic or recurrent and can impair an individual's ability to perform daily tasks. At its worst, depression can lead to suicide, which results in about 850,000 lost lives every year worldwide (6). Depression is not isolated to any one region or group. A recent study of 18 high and low-to-middle-income countries was performed by Bromet and colleagues (2011), using the World Mental Health Survey Initiative (13). They found that the average lifetime and 12-month prevalence estimates of major depressive episodes were 14.6% and 5.5% respectively in the ten high-income countries, and 11.1% and 5.9% respectively in the eight low- to middle-income countries (13).

According to Statistics Canada's last mental health survey cycle (2002), about 2.6 million adults aged 18 and older were suffering from a depressive disorder; this was over 10% of the Canadian population at the time of the survey (14). The percentage of Canadians reporting life-time diagnosed mood disorder rose from 5.3% in 2003 to 6.3% in 2009. Consistent with other studies, women reported significantly higher levels of depressive disorders than did men (7). In Alberta, lifetime prevalence of depressive disorders has been estimated to be 9% in the population (15) (see Figure 1).

Figure 1: Mental Disorders in Alberta – Lifetime Prevalence (15)



Diagnosis of Depression

The diagnosis of depression is mainly by clinical presentation based on a patient's medical history and physical examination. The patient may present with a variable set of symptoms (see Table 1). Diagnosis of depression is based on the criteria of a number of classification systems. In North America, the most commonly used system is the Diagnostic and Statistical Manual, 4th Edition (DSM-IV) (16). Other classification systems used to diagnose depression are the International Classification of Diseases (ICD), 9th or 10th Editions (17), and the Research Diagnostic Criteria (RDC) (18). The criteria for diagnosis of depression according to the DSM-IV is provided in Table 1 (16, 19).

Table 1: Criteria for diagnosing depression, based on the DSM-IV (16)

A. At least five of the following symptoms have been present during the same 2-week period and represent a change from previous functioning: at least one of the symptoms is either 1) depressed mood or 2) loss of interest or pleasure.

1. Depressed mood most of the day, nearly every day, as indicated either by subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated either by subjective account or observation made by others)
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or specific plan for committing suicide

B. The symptoms do not meet criteria for a mixed episode.

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
D. The symptoms are not due to the direct physiological effects of a substance (e.g. a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).
E. The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

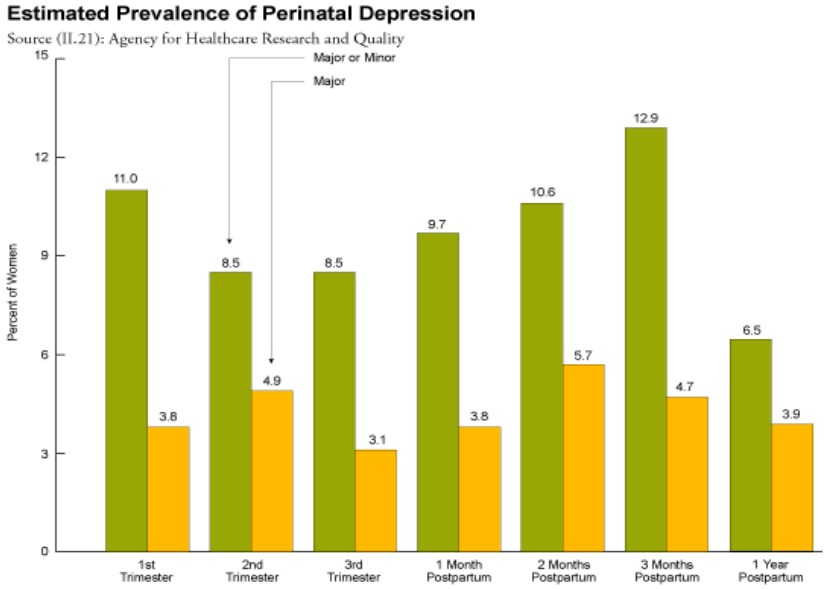
Diagnosis usually requires an in-depth personal interview with a psychiatrist or psychologist. However, a number of instruments have been developed and are available to family physicians to screen for depression in patients. The screening tools are useful when an extensive personal interview by a specialist is not possible. Some of the more widely used, validated screening tools are the General Health Questionnaire, the Beck Depression Inventory, the Symptom Checklist, and the Inventory of Depressive Symptoms (19). Screening tools to assess depression in specific groups (e.g. geriatric or obstetric populations) are also available. The Edinburgh Postnatal Depression Scale (EPDS) has been the most commonly used screener in the obstetric population to assess maternal depression (20). The EPDS will be discussed in detail in the Research Methods section.

Perinatal Depression

As stated, women are particularly vulnerable to developing depression, especially during pregnancy and following delivery. Research has focused primarily on postpartum depression;

however, it is now recognized that antenatal depression is just as frequent (perhaps more) as postpartum depression, and the two may be a continuum reflecting an underlying chronic condition among women during pregnancy and in the postpartum period. Data from the U.S. show that the prevalence of perinatal depression is bimodal, peaking in the first trimester, then again around 12 weeks post-delivery (11) (see Figure 2). Thus, perinatal depression is not an isolated event, but could be a continuum of undulating mood throughout pregnancy and in the postpartum period.

Figure 2: Estimated Prevalence of Perinatal Depression (21)



A recent review by Marcus for the Toronto Motherisk program reported that "recent studies suggest that 10% of gravid women meet criteria for major depression and up to 18% show elevated depressive symptomatology during gestation" (22). Overall, prevalence ranged between 10%-15% in adult women, depending upon the diagnostic criteria, timing of screening and screening instruments used. However, women with a prior history of depression were more likely to have a reoccurrence during pregnancy or in the postpartum period, with estimates

ranging from 25%-50%. Furthermore, women who had depression with psychotic features were also more likely to have recurrent episodes of perinatal depression, and 50-70% of women with a prior episode of postpartum affective psychosis may be at risk for recurrence of postpartum psychosis as well (22). In the Canadian Maternal Experience Survey (MES) report, 16.1% of women scored $\geq$ 10 on the EPDS (23). Women who scored $\geq$ 10 were more likely to be younger age (15–19 years), to have less than high school education, and to be at or below the low cut-off (< $40,000) for household income (23). Furthermore, 15.5% of women reported having been prescribed antidepressants or diagnosed with depression before their pregnancy (23).

Etiology of Depression

Because the diagnosis of depression is based on a variable set of symptoms, depression has been viewed not as a singular disease, but as a syndrome encompassing a spectrum of mood symptoms with multiple causes and possibly multiple patho-physiologies (24). One possible mechanism in the pathophysiology of maternal depression and the impact on fetal development is cortisol. The effect of maternal depression on birth outcome and fetal development has been presented in Chapter 4 (Manuscript 2); the effect of cortisol on birth outcome and fetal development will be discussed in the next section, *Effect of cortisol on fetal development*. The cause of depression is likely multifactorial, involving both genetic and environmental factors (25). Craddock and Forty (2006) reviewed linkage, genetic, family, and twin studies and found evidence implicating specific genes with regard to schizophrenia and mood disorders (25). The evidence of a genetic association in depression is not as strong as the evidence for bipolar disorder or schizophrenia; however, the literature is growing rapidly. One of the most interesting findings to emerge to date is the report of interaction between a functional variant at the

serotonin transporter gene and the occurrence of life events in early adulthood leading to the susceptibility of developing depression (25).

The serotonin transporter gene promoter polymorphism (5HTTLPR) has been implicated as a genetic susceptibility factor for depression. A study by Neumeister and colleagues (2002) examined the behavioral responses to tryptophan depletion in healthy women with and without a first-degree family history of depression and examined the relationship to 5HTTLPR alleles (26). They found the s-allele (but not the l-allele) of the 5HTTLPR gene and a positive family history of depression was additive risk factors for the development of depression in response to tryptophan depletion. This finding was confirmed in a study by Cervilla and colleagues in a large cohort (the PREDICT study) that showed the s/s 5-HTTLPR genotype increasing the risk for depression, independent of age, sex, or family history of psychological problems among first degree relatives and presence of comorbid generalized anxiety disorder (27). However, the 5HTTLPR gene has not been studied in perinatal depression.

It is well known that genetic susceptibility alone does not confer disease, as epidemiological data have shown that only about 40% to 50% of the risk for depression is linked to genetics (24). A prospective study by Laucht and colleagues looked at the interaction between the 5-HTTLPR gene and environmental adversity in relation to mood and anxiety psychopathology (28). Their results showed that depression and anxiety were strongly associated with both family adversity and stressful life events. As well, individuals with the l/l genotype (not the s/s genotype, as found in previous studies) of 5-HTTLPR who were exposed to high family adversity displayed significantly higher rates of depressive or anxiety disorders, and had more depressive symptoms than those without either condition (28). The authors concluded that a possible reason for the l/l genotype finding might be attributed to heterogeneity in depression

phenotypes and environmental adversity (28). The interplay of gene-environment was further explored by Rethorst and colleagues who examined how the association of the 5-HTTLPR genotype and depressive symptoms was moderated by physical activity. Their results showed significant interaction between 5-HTTLPR and physical activity ($p = .010$), where individuals with low levels of physical activity and at least one s allele had significantly higher levels of depressive symptoms compared to l/l individuals ($p = .011$) (29).

The 5HTTLPR genotype is clearly one of the variants associated with depression, but the results are not yet conclusive. To date, genetic studies on mood disorders have yet to provide conclusive evidence of specific susceptibility genes or their pattern of inheritance (30). It is beyond the scope of this literature review to provide an in-depth discussion on this topic, but it is evident that there is high degree of genetic variability, and the degree of interplay amongst the different genetic and environmental factors requires further exploration. The complex nature of the disease suggests that there are likely multiple genes and gene interactions, as well as gene-environment interaction (as emerging through the field of epigenetics) (31). A review by Corwin and colleagues examined three categories of genes associated with depression, and reported that conclusions were mixed on the contribution of genetic polymorphisms to the development of depression (31).

Numerous environmental factors have been linked to depression. Some of the known risk factors include gender (i.e. being female), low socio-economic status (SES) (e.g. financial insecurity), major life events and stresses (e.g. divorce, past physical and/or sexual abuse), chronic diseases (24), and lack of social support (32, 33). The prevalence of these risk factors were examined in the MES , which asked women about their perceived stress level the year prior to the birth of their baby, as well as the level of support they received during pregnancy (23). The

MES found that women who experienced more stressful events tended to be multiparous, younger age (15–19 years), had less than high school education, and lived in a household at or below the low income cut-off (<$40,000). The three most common stressful events cited by the women were 1) moving, 2) sick (hospitalized) family member, and 3) frequent arguments with a spouse (23). The level of support also varied by province and territory, with 15.0% of women in Nunuvut and 2.2% in Newfoundland-Labrador reporting receiving support "little or none of the time" (23). A prospective study by Collins and colleagues reported better outcomes for mother (e.g. labour progress) and child (e.g. Apgar scores) in a sample of low-income pregnant women with higher quality support (i.e. amount, quality and network resources) compared to those with little or no support (34). This finding is supported by a study that found perceived social support buffered the effects of perinatal depression, resulting in better birth outcomes (35).

As stated, lower SES may also confer risk for poor mental health. For example, the Young Finn study explore the impact of SES on depression by following a cohort of adolescents and young adults for 27 years (36). In 2007, when the subjects were in adulthood (age 30 to 45), the study found those who were younger, male, had higher parental occupational grade but not parental income and lower negative emotionality in childhood exhibited lower levels of depressive symptoms (36). Also in adulthood, those with a higher socioeconomic position and income had lower levels of depression (36). Low SES (thus, financial insecurity) may be directly related to food insecurity, which is another variable that has been associated with poor physical and mental health outcomes. Vozoris and Tarasuk (2003) analyzed data from the 1996/1997 National Population Health Survey, and found about 4% (1.1 million) of Canadians lived in food insufficient households; these individuals reported being 3.5 times more likely to suffer from major depression than those living in food sufficient households (37). Another study by Che and

Chen analyzed data from the 1998/99 National Population Health Survey and the Food Insecurity Supplement, and found households with food insecurity were significantly more likely to report poor/fair health (OR = 3.2, 95% CI = 2.6 – 4.0), multiple chronic conditions (OR 2.2, 95% CI = 1.8 – 2.7), obesity (OR = 1.5, 95% CI = 1.2 – 1.8), distress (OR = 3.2, 95% CI = 2.7 – 3.8), and depression (OR = 3.7, 95% CI = 2.9 – 4.7) (38). A U.S. study found women from households with food insufficiency met the criteria for major depression significantly more often than women without food insufficiency, after controlling for other risk factors (i.e. sociodemographics, number of children and adults in the household, marital status, and household income) associated with depression (39).

The role of nutrients, in particular micronutrients such as vitamins, minerals, and essential fatty acids, on depression is gaining increasing attention by the research and medical fields. A detailed discussion of how nutrient deficiency may be a risk factor in depression, and its role in the treatment of depression, is presented in the section below "*the role of nutrition on depression*".

Effect of cortisol on fetal development

The pathophysiology of maternal depression with regard to the hypothalamic-pituitary-adrenal (HPA) axis and the role of cortisol is presented in Chapter 4 (Manuscript 2). Cortisol may be the causal factor in pregnancy and birth complications, including the delivery of premature and low birthweight babies (40, 41, 42). Elevated cortisol and corticotropic releasing hormone (CRH)), seen in women with depression (43-45), is the proposed mechanism by which cortisol exerts its effect on fetal development (46, 47). It has been hypothesized that the elevated cortisol levels observed in prenatal depression has led to increased fetal activity, delayed prenatal growth leading to prematurity and low birth weight (46, 47). Animal models have shown that

elevated maternal cortisol may affect fetal growth by both changing the placental environment and by directly crossing the placenta (48). It has been proposed that elevated CRH in the placenta may lead to vasodilatation, and uterine artery constriction, thus reducing blood flow to the fetus. Reduced blood flow to the fetus, which then restricted oxygen and nutrient delivery, has been associated with birth complications including prematurity (40).

A number of studies have examined the association between maternal cortisol levels with birth and fetal outcomes. A review by Field and colleagues (46) reported that elevated prenatal maternal cortisol was the strongest predictor of poor birth and neonatal outcomes. Furthermore, the biochemical and physiological profiles of babies born to depressed women were very similar to their mothers' prenatal biochemical/physiological profiles including elevated cortisol, lower levels of dopamine and serotonin, as well as greater EEG activation in the right frontal area of the brain and lower vagus nerve activity. Another study by Field and colleagues (46) found cortisol levels were more accurate in predicting prematurity and low birth weight than depression scores using the Center for Epidemiological Studies – Depression Scale (CES-D). As well, fetuses of women with elevated cortisol levels experienced growth delays in head circumference, abdominal circumference, biparietal diameter and fetal weight, and increased fetal activity (e.g. fetal movement) (47) as measured by ultrasound.

A study by Hompes and colleagues (49) explored the influence of maternal cortisol (from saliva samples) and emotional state (using psychological assessment) during pregnancy on fetal intrauterine growth (from ultrasound). Their results showed basal cortisol levels significantly predicted the variance of weight and body mass index at birth. A study by Bolton and colleagues (50) investigated cortisol as a mediating mechanism in the association between maternal distress and birth outcomes. The results showed maternal cortisol levels were associated with birth

weight and body length at birth, even after controlling for gestational age, parity, pre-pregnancy BMI, smoking, and infant's sex. Newborns of mothers with higher cortisol levels in pregnancy had lower birth weights and were shorter at birth. Bolton and colleagues concluded that maternal cortisol levels in pregnancy influence intrauterine growth and may be a better predictor for birth outcome than perceived stress (50). A prospective cohort study by Goedhart and colleagues (51) examined the association between maternal cortisol levels (from serum), as well as maternal psychosocial well-being (from questionnaire), with offspring birthweight and small for gestational age (SGA) risk. The results indicated maternal cortisol levels were negatively related to offspring birthweight ($B=-0.35$; $p < 0.001$) and positively to SGA (OR = 1.00; $p = 0.027$), but after adjusting for gestational age at birth, infant gender, ethnicity, maternal age, parity, BMI, and smoking, the initial effects were no longer statistically significant (51). However, the authors conducted post hoc analysese, and found a moderation effect by time of day; that is, women who provided a morning blood sample (before 9 am) and who had higher maternal cortisol levels were independently related to lower birthweights ($\beta = -0.94$; $p = 0.025$) and a higher SGA risk (OR = 1.01; $p = 0.032$) (51). They also reported that maternal psychosocial problems were not associated with cortisol levels (51).

Treatments for Depression

Present conventional treatments for depression include antidepressants and psychotherapies. The modern generation of antidepressants is claimed to have the advantage of fewer side effects which leads to better patient adherence (19); however, they still carry a risk for varying degrees of adverse events, as well as the potential for overdose and significant drug-drug interaction with other medications (19, 52). Additionally, the use of antidepressants during pregnancy has the added caveat of the potential adverse effects on the developing fetus that may

not be immediately obvious (53). A prospective case-control study by Lewis and colleagues found infants exposed to antidepressants *in utero* were eight times more likely to born prematurely, had significantly lower birth weight and were smaller in length and head circumference than non-exposed infants, and at 1 month, the difference in weight in the exposed group became significantly greater than the control group (54). Einarson and colleagues analyzed data from the Motherisk database and found more preterm births (OR = 1.7, 95% CI = 1.18 to 2.45), and greater number of smaller than gestational age (SGA) babies (OR = 1.19, 95% CI = 0.86 to 1.64) in a group of women who took antidepressants during pregnancy compared to controls (55). However, there was no difference in birth weight between the groups in this study (55). A systematic review by Simoncelli and colleagues reported that studies on the teratogenic effects of common antidepressants were variable and inconclusive (56). While some antidepressants did not pose a major teratogenic risk, others medications, such as paroxetine, was linked to an increase in the risk of cardiovascular malformations in the foetus. The impact of prenatal exposure to antidepressants on prematurity and low birth weight remain controversial, and most studies evaluating these outcomes have been limited by small sample size and inadequate reference groups. Simoncelli and colleagues concluded the information on the long-term effects of gestational antidepressant use on child development, while starting to emerge, was too limited to determine risk (56).

According to Nestler and colleagues (2002), treatment for depression remains sub-optimal, with only about 50% of patients achieving complete remission in the short term, and effective therapy strategies remaining unclear (24). When the patients are women who are pregnant or lactating, pharmaceutical treatment is further complicated by the potential risk to the

developing foetus or newborn (57). For example, all antidepressants are excreted in breast milk; however, the long term deleterious effects on the infant have yet to be well studied (52, 57).

Due to limited success and side effects of conventional medications, many people have turned to nonconventional therapies to treat their depression. One study (58) found that up to 54% of women with depression reported using nonconventional therapies; these included botanicals, homeopathy, massage therapy, meditation, and dietary supplements, such as vitamins, minerals and essential fatty acids (59). The efficacy of these therapies has yet to be determined; however, there are a growing number of studies supporting the role micronutrients in depression (60-64). For example, cohort studies have examined the potential link between nutrient deficiency and depression (65, 66), while intervention studies have examined the ability of nutrients such as omega-3 fatty acids to treat depressive symptoms (67, 68). The role of micronutrients (i.e. vitamin, mineral, essential fatty acids) in depression, in particular, perinatal depression, is the focus of the first component of this thesis.

The Role of Nutrition in Depression

Pregnancy presents unique stresses that challenge overall physical and psychological adaptation. It is a time in which variations in nutrient intake affect health outcomes for both the mother and foetus (69). Women during pregnancy and lactation are particularly vulnerable to the adverse effects of inadequate intake of nutrients (in particular micronutrients such as fatty acids, vitamins, and minerals) on mood because those are periods of increased nutrient requirements (70, 71). It has been proposed that depletion of nutrient reserves throughout pregnancy and a lack of recovery postpartum may increase a woman's risk for maternal depression (63). Deficiency of some individual nutrients (e.g. vitamin D) has been found to be a risk for depression (72), while

intervention studies have found other nutrients (e.g. folic acid) can improve depressive symptoms or reduce the risk of developing depression (73).

A number of nutrients are getting increasing research attention because of their potential role in brain function and mental health. One of the nutrients that has been strongly associated with mental health and treatment for depression is the omega-3 fatty acids (74). Lipids (by dry weight) make up about 50% to 60% of the adult brain, and about 35% are comprised of unsaturated fatty acids, namely docosahexaenoic acid and arachidonic acids being the highest concentration (75). The Agency for Healthcare Research and Quality (AHRQ) conducted a systematic review in 2005 of the scientific medical literature to assess the effects of omega-3 on mental health (75).,They reported that a majority of the studies examined the use of omega-3 for the treatment of schizophrenia and depression, with mixed results showing some short term benefits in both conditions, but overall inconsistencies with supplement dosage, small sample sizes, difference in outcome measures, and other methodological issues, rendering inconclusive the implications for clinical significance . However, a large number of observational studies and clinical trials continued to explore the potential effects of omega-3 for the prevention and treatment of depression (76, 77), often with mixed findings. Table 2 is an updated summary of the observational and experimental studies on omega-3 and depression not covered in manuscript 2 (Chapter 4).

Other nutrients, such as folate, vitamin D, iron, selenium, and zinc, have also been implicated in the development of (and treatment for) depression. Folate is known to be necessary for the proper biosynthesis of serotonin, a monoamine neurotransmitter that has implicated in depression (78). Folate is integral to nucleotide synthesis, DNA integrity and transcription, and folate's role in brain development has also been established through its well-demonstrated role in

the formation of the neural tube after conception (78). Folate may also be required for methylation of phospholipids in neuronal membranes and have an impact on membrane-bound receptors, second messenger systems and ion channels (79). A review on folate by Fava and Mischoulon reported that different formulations of folate (folic acid, 5-methyltetrahydrofolate (5-MTHF), and folinic acid) showed differing effects on symptoms of depression (73). For example, the 5-MTHF formulation was effective only on depressed patients with normal and low folate levels, while adjunctive folinic acid reduced depressive symptoms in patients who were partially responsive or nonresponsive to a selective serotonin reuptake inhibitor (61, 73).

Vitamin D has been posited to be neuroactive steroid that can affect brain neurochemistry and brain function, and low levels of vitamin D has been linked to depression (80). A review by Bertone-Johnson of nine epidemiological studies was inconclusive regarding vitamin D status and its association with the occurrence of depression (72). In her review, Bertone-Johnson found that one clinical trial showed significant evidence of modest improvement in Beck Depression Index (BDI) scores after 1 year of vitamin D supplementation, while other studies (all cross-sectional) did not show any association between vitamin D and depression (72).

A number of minerals have also been implicated in mental health and depression. Iron is vital to brain function, and the brain places demands on iron availability that are regional, cellular, and age sensitive (81). In women of reproductive age, studies have reported neuropsychological consequences of iron deficiencythat included changes in cognitive performance, emotions, and behavior (82). Selenium is an antioxidant that is recognized for its role in brain function. The functions of selenium are believed to be carried out by selenoproteins, although their roles in neuronal function remains to be fully defined (83). Selenium depletion in animal studies has been associated with decreased activities of Se-dependent enzymes, leading to

cell loss and changes to brain function (84). Zinc has been shown to be essential for brain growth and function, and in human studies, subclinical zinc deficiency led to changes in brain function, including decrease in motor skills (e.g. tracking), reasoning, memory, and attention (85). Takeda proposed that zinc operates via the hippocampus, and changes in hippocampal function may be a risk factor for depressive symptoms under chronic stress circumstances (86). Table 3 is an updated summary of the observational and experimental studies on the vitamins and minerals associated with depression not covered in manuscript 2 (Chapter 4).

In summary, the literature reviewed here suggests that a key to the prevention and treatment of perinatal depression may be to improve overall nutrition in pregnant women. Further research is warranted to establish whether nutrition levels are associated with perinatal depression in women, as well as the role of nutrients in foetal/child cognitive and behavioural development.

Objectives

The *primary objective* is to assess whether there is a relationship between maternal nutrition and perinatal depression.

The *secondary objective* is to explore whether maternal nutrition is associated with the offspring's behavioural and cognitive development.

Thesis Outline

This manuscript-based thesis is composed of two parts. Part 1 addresses the primary objective stated above. Chapter 1 provided an in-depth review of the literature and Chapter 2 discussed the methods, adding details which were not fully covered in the manuscripts. At the time of the development of the thesis proposal, and the subsequent development of Manuscripts 1 and 2, research was starting to emerge on the association between nutrition and depression.

Thus, Manuscripts 1 and 2 are comprehensive reviews and synthesis of the global literature on the role of nutrition in brain function and on mental health, namely depression and perinatal depression. Manuscript 3 was developed with primary data from the Alberta Pregnancy Outcomes and Nutrition (APrON) study, with the aim of examining the role of micronutrients (from supplements) on postpartum depression in a pregnancy cohort. The three manuscripts comprise the next three chapters (3 to 5) of the thesis.

To further understand the impact of nutrition on brain function and development, Part 2 of this thesis addresses objective 2, which is to examine the role of micronutrients in the area of brain function in children (Chapter 6). Chapter 7 examines whether maternal supplement had any impact in the neurocognitive development in the offspring. Chapter 8 is a general discussion on the common theme that ties the publications together: how micronutrients can affect mood in pregnant women and brain development in children. The thesis concludes with recommendations for future research.

Table 2: Updated summary of studies on omega-3 and maternal depression

Study	Design & sample size (n)	Measurement	Outcome	Limitations
Browne, 2006 (87)	Prospective cohort, n=80 postnatal women, 41 diagnosed with depression, 39 controls	EPDS & BDI-II; diagnosis with the CIDI; single FFQ collected during pregnancy; postnatal venous blood (fasted) in EDTA tubes	Prenatal fish consumption was not predictive of PPD, & post-natal omega-3 status was not associated with PPD	Single FFQ and blood sample collected on fish intake & PUFA status; majority ate non-oily fish, which was not separated from oily fish consumption
deVriese, 2003 (64)	Cross-sectional study, n = 48, 10 with PPD, 38 without	Blood samples extracted shortly after delivery & assayed for serum phospholipids and cholesteryl esters; DSM-interview assessed for depression	Fatty acid composition was lower in women with depression than those not depressed	Temporality not established; i.e. if low fatty acid precedes depression or vise versa
Doornbos, 2009 (88)	RCT, n = 119; placebo, DHA (220 mg) or DHA+AA (220 mg each) from week 16 of pregnancy till 3 months postpartum	Plasma fatty acid analyzed at 16 & 36 weeks of pregnancy; EPDS weeks 16 & 36 of pregnancy and 6 weeks postpartum, a "blues" questionnaire at one week postpartum	No difference in EPDS scores or "blues" questionnaire; red blood cell DHA, AA and DHA/AA ratio did not correlate with EPDS or blues scores	Dose of DHA may be too low; small sample size for 3 arm trial; women from general population, not a diagnosed group, thus "intervention" is not suitable for RCT
Freeman, 2006 (67)	RCT, PPD subjects were randomized to 0.5 g/day (n = 6), 1.4 g/day (n = 3), or 2.8 g/day (n = 7) for 8 wks.	EPDS & HRSD before and after treatment	Significant within group difference in pre- & post-treatment scores, ↓ 51.5% for EPDS, ↓48.8% for HRSD; no significant between group difference	Small sample size and lack of placebo group
Golding,	ALSPAC cohort	Questionnaire that	Women consuming	Use of single

Study	Design & sample size (n)	Measurement	Outcome	Limitations
2009 (89)	study, n = 14,541 pregnant women	included symptoms of depression and FFQ at 32 weeks gestation	no omega 3 from seafood were 50% more likely to have high levels of depressive symptoms compared to those eating 1.5 g omega-3 per week (adjusted OR = 1.54; 95% CI = 1.25-1.89)	dietary measure
Llorente, 2003 (68)	RCT, breastfeeding moms randomized to DHA (200mg/d) (n=44) or placebo (n=45) for 4 months	Plasma phospholipid fatty acid patterns; self rated questionnaire; structured interview for depression in a subgroup	DHA content was 8% higher in the treatment group & 31% lower in the placebo group at 4 months; no difference between the 2 groups on self rated or diagnosed depression	Small sample size; short duration; low DHA dosage
Makrides, 2010 (90)	RCT, double blind, n = 2399 Australian women < 21 week gestation, given 800 mg/d of DHA fish oil or placebo vegetable oil	EPDS at 6 weeks or 6 months postpartum, score > 12 classified as "High levels of depressive symptoms"	No difference in "high level" scores between the two groups at 6 months, adjusted relative risk = 0.85; 95% CI, 0.70-1.02; p = .09)	Dose of DHA too low; the women were from general population, not a diagnosed group; blood levels not measured, and no dietary measure to assess if women were getting DHA from dietary sources
Miyake, 2006 (91)	Prospective cohort, n=865 pregnant Japanese women	EPDS; self-administered diet history questionnaire during pregnancy	No significant association found between dietary fish / fat intake and PPD	Wide range (2 – 9 mos) for postnatal screening; Single self administered semi-quanti-

Study	Design & sample size (n)	Measurement	Outcome	Limitations
				tative dietary questionnaire.
Otto, 2003 (92)	Prospective cohort, n=112 pregnant Dutch women	Venous blood samples were collected at week 36 of pregnancy, after delivery, and 32 weeks postpartum. PPD assessed retrospectively at week 32 after delivery, using EPDS	Omega 3:6 ratio was significantly lower in the 'possibly depressed' group (EPDS $\geq$ 10) compared to the not likely-depressed group (EPDS <10)	Covariates associated with depression unknown
Peet, 1998 (93)	Cross-sectional study, n=30, 15 depressed, 15 controls	Fatty acid composition in red blood cells	Depressed patients had significantly lower omega-3 PUFA, DHA	Temporal relationship unknown; no control for covariates such as smoking, drug use, etc.
Strøm, 2009 (94)	DNBC, n = 54,202 Danish women	Clinically identified cases of depression up to 1 yr postpartum from registry; fish intake at mid-pregnancy assessed with FFQ	No association was observed between fish intake & risk of PPD with adjusted OR = 0.82 (95% CI: 0.42, 1.64)	Use of single diet measure; PPD tends to be underdiagnosed, thus reported rate of 1.9% is lower than prevalence in the literature
Su, 2003 (95)	RCT, double blinded, placebo controlled; omega-3 PUFAs (9.6 g/day), for 8 weeks, n=22 depressed patients: 12 in treatment group, 10 placebo	HRSD	Patients in the omega-3 PUFA group had a significantly decreased score on the 21-item HRSD compared to the placebo group	
Su, 2008 (96)	RCT, 8-week, double-blind, placebo-	HRSD, EPDS, BDI	Compared to the placebo group, the omega-3 group had	

Study	Design & sample size (n)	Measurement	Outcome	Limitations
	controlled trial comparing omega-3 PUFAs (3.4 g/d) with placebo in pregnant women (n = 24) with major depressive disorder (DSM-IV criteria)		significantly lower HRSD scores at weeks 6 (p = .001) and 8 (p = .019), a significantly higher response rate (62% vs. 27%, p = .03); also significantly lower depressive symptom ratings on the EPDS and BDI at study endpoint	

Key: RCT = randomized control trial; EPDS = Edinburgh Postpartum Depression Scale; BDI-II = Beck Depression Inventory (version 2); CIDI = Composite International Diagnostic Interview; PPD = postpartum depression; FFQ = food frequency questionnaire; HRSD = Hamilton Rating Scale for Depression; PUFA = polyunsaturated fatty acid; DHA = Docosahexaenoic acid; AA = Arachidonic acid; ALSPAC = Avon Longitudinal Study of Parents and Children; DNBC = Danish National Birth Cohort; DSM-IV = Diagnostic and Statistical Manual (4th Edition); EDTA = Ethylenediaminetetraacetic acid

Table 3: Updated summary of studies on vitamins & minerals and depression

Study	Design & sample size (n)	Nutrient(s)	Measurement	Outcome
Albacara, 2010 (97)	Prospective cohort, depression-free Spanish postpartum women (n=729)	Iron	EPDS given at 48 hours, 8 weeks & 32 weeks postpartum, diagnostic interview using DSM criteria; blood sample obtained 48 h after delivery	65 women (9%) had PPD @ 32 week postpartum; lower ferritin associated with PPD (OR=3.73, 95% CI: 1.84–7.56; p=0.001)
Amani, 2010 (98)	Case-control, 20–25-year-old female students; 23 identified with moderate and severe depression, and 23 healthy age matched controls	Zinc	BDI, 12-item semi-quantitative FFQ, and 24-h food recall, blood sample	Daily zinc intake & serum levels were 1/3 lower in cases relative to controls; serum zinc levels inversely correlated to depression scales
Bertone-Johnson, 2011 (99)	RCT, n= 36,282 post-menopausal women.	400 IU of vitamin D3 + 1,000 mg calcium	Burnam scale and antidepressant use	No relationship found for vitamin D3 + calcium supplement & depression or antidepressant use in this group
Ganji, 2010 (100)	NHANES III, cross-sectional survey, n= 7970 non-institutionalized US residents, aged 15-39 y	Vitamin D	Diagnostic Interview Schedule, serum vitamin D concentrations	Depressive episodes in persons with serum vitamin D ≤ 50 nmol/L were significantly higher than those with serum vitamin D ≥ 75 nmol/L (OR = 1.85 P = 0.021)
Irmisch, 2010 (101)	Case control, n= 88 depressive in-patients and 88 volunteers from the general population matched for age-group and gender	Zinc and fatty acids	BDI and HDRS, blood samples	FA composition in serum significantly differed between depressive and healthy persons: Depressive patients had higher stearic & AA & lower EPA

Study	Design & sample size (n)	Nutrient(s)	Measurement	Outcome
	(each 32 men and 56 women, 21–70 years)			and DHA concentrations; Zn significantly correlated with the severity of depression (r = 0.26; P = 0.034)
Maserejian, 2011 (102)	Cross-sectional, observational study from Boston Area Community Health survey, n=2163 women, and n=1545 men	Zinc	Validated FFQ & abridged validated CESD scale	Low zinc intake associated with depressive symptoms in women (e.g. comparing 1 vs. 4 quartiles, OR=1.76, 95% CI = 1.26, 2.45; P-trend=0.004), but *not* in men
Miyake, 2006 (66)	Prospective cohort, n= 865 Japanese women	folate and vitamins B12, B6, and B2	diet history questionnaire; EPDS at 2 & 9 months postpartum	121 subjects (14.0%) scored $\geq$ 9 on EPDS classified as "depression"; subjects in 3rd quartile for B2 had decreased risk of depression (OR = 0.53, 95% CI: 0.29-0.95, P for trend=0.55); no associations with folate, B12 or B6
Mokhber, 2011 (103)	RCT, n= 166 primigravida pregnant women in the first trimester randomized to receive 100 mg of selenium (n = 83) or a placebo (n = 83) per day until delivery	Selenium	EPDS & serum selenium concentrations were measured at baseline and at the end of study.	Mean EPDS score in the selenium group was significantly lower than that of the control group (p = 0.05)
Murphy, 2010 (104)	Exploratory, descriptive study using a convenience	Vitamin D	EPDS, seven monthly visits postpartum	Significant relationship over time was found between low

Study	Design & sample size (n)	Nutrient(s)	Measurement	Outcome
	sample, n= 97 postpartum women			25(OH)D levels and high EPDS scores
Pasco, 2012 (105)	Nested case-control study, women from the Geelong Osteoporosis Study, compared high vs low(<8.9 µg/MJ/day) selenium intake	Selenium	Major depressive disorder , identified using the Structured Clinical Interview for DSM-IV-TR	Adjusting for age and SES, compared with a high selenium intake, those with low intake were almost 3 times more likely to present with major depressive disorder; (OR 2.95, 95%CI 1.00-8.72)
Rayman, 2006 (106)	Placebo-controlled RCT, 501 UK participants aged 60–74 randomly allocated to receive 100, 200 or 300 mcg selenium/d as high-selenium yeast or placebo yeast	Selenium	POMS-BI questionnaire, "quality of life" SF-36 questionnaire, & plasma selenium measured at baseline & 6 months	After six months of supplementation, mean (SD) total mood scores increased for placebo & 100 mcg groups & decreased for 200 & 300 mcg groups, but NOT statistically significant (p=0.86)
Roy, 2010 (107)	Prospective cohort , n= 2030 pregnant women from the Prenatal Health Project, London, Ontario.	Zinc	CES-D, FFQ	Social disadvantage, higher stress, and lower zinc intake were associated with higher CES-D score. Every 1-point increase in stress score was associated with a 1-point increase in CES-D score. Being in the lowest quintile of zinc intake was associated with a 1-point increase in CES-D score
Sawada & Yokoi, 2010 (108)	RCT, n= 30 Japanese women, randomized in	Zinc	POMS, blood sample	Women who took MV and Zn showed a significant reduction

Study	Design & sample size (n)	Nutrient(s)	Measurement	Outcome
	equal numbers to multivitamins (MVs) or MV + 7 mg Zn daily for 10 weeks			in anger–hostility score (P = 0.009) & depression–dejection score (P = 0.011)
Watanabe, 2012 (109)	Cross-sectional study, n= 141 Japanese women age 18-28	Folate	CES-D scale, diet history questionnaire, blood samples of folate & homocysteine	Low folate intake (<240 mcg/d) was significantly associated with depressive symptoms; folate intake at or above RDA decreased the likelihood of depressive symptoms (OR = 0.22; 95% CI = 0.11-0.56; *P* < *0*.001)

Key: RCT = randomized controlled trial, EPDS = Edinburgh Postpartum Depression Scale, BDI = Beck Depression Inventory, CIDI = Composite International Diagnostic Interview, FFQ = Food Frequency Questionnaire, EDTA = Ethylenediaminetetraacetic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid, AA = Arachidonic acid, HRSD = Hamilton Rating Scale for Depression, ALSPAC = Avon Longitudinal Study of Parents and Children, DNBC = Danish National Birth Cohort, NHANES = National Health and Nutrition Examination Survey, CES-D = Center for Epidemiologic Studies Depression, 25(OH)D = 25-hydroxyvitamin D, POMS-BI = Profile of Moods States - Bipolar Form, SF-36 = Medical Outcome Study Short Form 36

CHAPTER 2: METHODS

Outline

This chapter outlines the intent of each manuscript, as well as the source of data used for each manuscript. The methodology was described within each manuscript, which was independently produced. That is, Chapters 3, 4 and 7 were reviews and the data came from the global literature. The methods for these chapters are detailed in the manuscripts, and thus will not be repeated here. The data for Chapter 5 was collected through the APrON Study. As APrON was a large longitudinal cohort study, much of the detail on its methodology could not be included in the manuscript. Thus, details of the methods used in the APrON Study (but not provided in manuscript 5) are elaborated in this chapter.

Chapter 3, Manuscript 1

*Kaplan B, **Leung B**. Multi-micronutrient supplementation for the treatment of psychiatric symptoms. Integrative Medicine: A Clinician's Journal, 10(3), Jun-Jul 2011*

Manuscript 1 was a review of nutritional supplements used in treating mental disorders, including depression. As presented in the background, a number of nutrients are involved in brain function, and have been associated with mental disorders, particularly depression. Findings from this review helped to focus the list of nutrients known to have possible effects on depression, and informed the list of nutrients that was analyzed in Manuscript 3. The methods for the literature review were detailed in the publication and will not be repeated here.

Chapter 4, Manuscript 2

***Leung BM**, Kaplan, B. Perinatal Depression: prevalence, risks and nutrition – a review of the literature; Journal of the American Dietetics Association, 2009. 109: 1566-1575*

Manuscript 2 assessed the global literature to determine the prevalence and risk factors, in particular nutrients, for perinatal depression. An update of the literature that was not available at the time of publication is provided in Tables 2 (Summary of studies on omega-3 and maternal depression), 3 (Summary of studies on vitamins & minerals and depression), and 8 (Summary of studies on nutrient inadequacies in pregnant women). The risk factors (including nutrients) known to be associated with perinatal depression were then used as predictors and covariates in the APrON study, and were included in the multivariate analyses for Manuscript 3. The methods for the literature review were detailed in the publication and will not be repeated here.

Chapter 5, Manuscript 3

***Leung MY Brenda**, Kaplan J Bonnie, Field J Catherine, Tough Suzanne, Eliasziw Misha, Gomez Fajer Mariel, McCargar J Linda, Gagnon Lisa, and the APrON Study Team. Prenatal micronutrient supplementation and postpartum depressive symptoms in a pregnancy cohort. BMC Pregnancy and Childbirth. 2013, 13:2. DOI: 10.1186/1471-2393-13-2.*

Manuscript 3 used data from the APrON study. Many details of the methodology and the measurement tools used for data collection were not provided in the manuscript due to space (word) limitations, and are expanded upon in this chapter. Thus, this thesis is an opportunity to provide additional details about the process and procedures for data collection, management and analyses used in the APrON study. Table 4 is a summary of the tools and timeline of data collection.

<u>Measurement tools</u>

1. Maternal Depression assessed by the Edinburgh Postnatal Depression Scale

The Edinburgh Postnatal Depression Scale (EPDS) is a 10-item scale measuring mood, is self-administered, and requires about five minutes to complete (see Appendix 1). The EPDS has

been used extensively worldwide, and has been evaluated for its psychometric rigour (20, 110-114). It has been found to have high sensitivity (83.6%) and specificity (88.3%) at a threshold score of 10 for identifying those at risk of, or potentially suffering from, either antenatal or postpartum depression (115). The EPDS has a moderate to good reliability and test-retest reliability and has a good to moderate correlation with other depression measures (111).

The EPDS has been used for clinical and research purposes and cut-off scores have been obtained through empirical assessment. These cut-offs provide rates of probable depression for referral or treatment, and can also be used to track the course of depression from antenatal to postpartum periods. As with other screening measures, sensitivity and positive predictive value may be variable when used in selected populations versus community populations (111, 112). Matthey and colleagues (116) reviewed the literature and recommended the EPDS *cut-off scores* from validated studies for English speaking women as follows: a) antenatally, a score of $\geq$ 15 = "probable major depression" and 13 – 14 = "at least probable minor depression", b) postnatally, a score of $\geq$ 13 = probable major depression", and 10 – 12 = "at least probable minor depression". For the purpose of the APrON study, a cut-off score of 10 for probable depression was used.

2. Maternal Anxiety assessed by the Symptom Checklist-90-Revised Anxiety Dimension Scale

The Symptom Checklist-90-Revised (SCL-90R) anxiety dimension is a ten-item scale that is part of the larger 90-item checklist. APrON was given permission by the SCL-90R publisher, Pearson Publishing Inc, to use the 10 item anxiety subscale, along with 15 buffer questions recommended by their statistician. This recommendation was to help avoid the potential of "response set" (i.e. tendency for respondents to answer all questions the same way) that could result in skewed data. This method of using the subscale has not been validated.

However, with the assent of the publisher, only the 10 items were analyzed; the 15 buffer questions were not included in the analysis. See Appendix 2 for the 10 anxiety items and 15 buffer questions.

For each item on the anxiety subscale, five possible responses were offered (not at all, a little bit, moderately, quite a bit, and extremely). Each response was assigned a numerical value of 0-4, with 0 indicating "not at all" and 4 indicating "quite a bit". We followed the Administration, Scoring and Procedures Manual (117) from the publisher, and calculated the raw scores by summing the values to the responses and then dividing by the number of items in the anxiety dimension. Thus a total of 4 (i.e. 40/10) was the highest possible score. No known cut-off scores were found in the literature for the SCL-90R, and we have found no studies that have used the anxiety subscale alone.

3. Perceived Social Support assessed by modified National Population Health Survey – Social Support

To assess perceived social support, questions from the National Population Health Survey (NPHS) – Social Support section (Statistics Canada, 1994/95 & 1996/97 cycles) (118) were used, with response options modified for the APrON study. The NPHS-Social Support section is comprised of four statements about having someone to "confide in", "count on in a crisis", "count on for advice", and " make one feel loved and cared for", with Yes/No response options. Guided by expert advice from team members, the APrON study kept the wording of the statements, but modified the response options to five possible answers: none of the time, a little of the time, about half of the time, most of the time, all of the time. Each response option was then assigned a numerical value, "none of the time" was assigned a value of 0 and ranged up to a value of 4 for "all of the time". By summing the scores of each statement, an overall score of 16

indicated the highest possible level of perceived social support, while an overall score of 0 was the lowest possible level of perceived social support. See Appendix 3.

4. Stressful Life Events assessed by Modified Stressful Life Events Questionnaire

Questions regarding stressful life events were derived from the Stressful Life Events Questionnaire (SLEQ) (119). Six items of the SLEQ were used in APrON's modified questionnaire based on the study by Bergman and colleagues (2007). In that study, Bergman reported that relational items had the strongest association with poor child outcome, as measured by the Bayley Scales of Infant Development (BSID) – 2nd Edition and the Mental Development Index (MDI) (119). In fact, the items pertaining to relationship strain explained "73.5% – 75.0% of the total variance of prenatal stressful events" on infant outcomes and fearfulness (119). The study found that items related to the woman's partner and family had the strongest effect (i.e. the most stressful) on pregnant women. Thus, it was postulated that this in turn had an impact on a woman's mental health and her ability to care for her child, leading to poorer child outcomes. This idea is supported by the existing literature on the impact of maternal stress on maternal mental health, resulting in poor birth and infant outcomes (120).

The six items from the SLEQ chosen for APrON had shown significant correlations with the child outcome measures by Bergman and colleagues (119). In addition, one question on sexual abuse prior to age 17 was added following the advice of the maternal mental health expert on the APrON team, Dr. Victor Pop. The modified SLEQ was developed by APrON researchers, and focused on three critical periods of the woman's life: before age 17 (three questions), 12 months before current pregnancy (seven questions), and during pregnancy (seven questions). The response options were No, event did not happen at (life period) or Yes, event has happened at (life period). Furthermore, if the women answered Yes, a follow up question asked "how much

did this affect you", with response options: not at all, somewhat, and a lot. See Appendix 4. For the purpose of this study, the answers are dichotomized to Yes or No, and the follow up response to the Yes questions were not analyzed, as this variable was only included as a covariate.

5. Personal Health History (physical and mental) assessed by questionnaire developed for the purpose of APrON

Questions on self reported mental health history and history of chronic conditions were developed through expert input from APrON team members. A list of the most common conditions was presented in a table format, and the women answered Yes or No to the question "Do you currently have this condition?". See Appendix 5.

6. Socio-demographic & Background Information assessed by questionnaire developed for the purpose of APrON

Questions on socio-demographic background, including age, marital status, education level, smoking/alcohol/drugs, BMI, and pregnancy history were derived from questions used in the NPHS and the Community Perinatal study. See Appendix 6.

7. Micronutrient Intake assessed by the Supplement Intake Questionnaire

Information on prenatal micronutrient supplement intake was collected using the Supplement Intake Questionnaire (SIQ) (see Appendix 7), developed for the purpose of APrON. The SIQ was pre-tested and validated with a group of pregnant women prior to use. Details pertaining to data collection using the SIQ were provided Chapter 5 (Manuscript 3).

Pretesting

The questions developed for the covariates were tested for face validity using a convenience sample of associates at the Behavioural Research Unit of the Alberta Children's Hospital prior to inclusion into the final questionnaire.

Data Collection Procedure

The women who participated in APrON were seen at the Behavioural Research Unit at the Alberta Children's Hospital in Calgary and at the Alberta Institute of Human Nutrition, University of Alberta, Edmonton. Once a woman agreed to participate in the study, she was sent a welcome email with a two page summary about APrON. A research assistant telephoned the woman to book the first appointment, and a confirmation email was sent prior to the visit, along with the following documents:

- Maternal consent form
- Maternal contact information form
- Paternal consent form
- First visit questionnaire
- Directions to get to the APrON clinic

Prior to the clinic appointment, the woman was sent another reminder email to bring the completed forms with her to the appointment.

At each visit, the women were interviewed by research assistants who were trained on the 24 hour food recall, anthropometric measurements, and other data collection activities. Blood samples were taken by a phlebotomist. The women also provided urine and breast milk samples when appropriate. Each appointment took about 25 to 30 minutes. A timeline of the data collection time points is outlined in (Table 4). The following summarizes the visit by time point:

1st Visit at APrON (designated either Time A or B, depending on trimester)

- Participant handed in finished forms;
- Participant went through 24hr food recall, bloodwork, anthropometric measurements, and provided a urine sample if needed;

- Participant took home: FFQ, Pre-Pregnancy Physical Activity questionnaire, and Dietary Changes questionnaire, as well as forms for father if father was participating, including the Paternal Consent form, Paternal Contact form, Paternal first questionnaire, and Paternal Family History form.

2nd Visit at APrON (designated as either Time B or C)

- Participant handed in the completed FFQ, Dietary Changes, and either Time B or C questionnaires mailed to them prior to this appointment
- Participant might also hand in paternal forms
- Participant went through 24hr food recall, bloodwork, anthropometric measurements, and provided urine sample if needed

3rd Visit at APrON (designated as Time C)

- Participant handed in completed Time C questionnaire and Family History form;
- Participant might also hand in paternal forms
- Participant went through 24hr food recall, bloodwork, anthropometric measurements, and provided urine sample if needed

Last Visit at APrON (designated Time E)

- Appointment date was set at 12 weeks post partum ± 2 weeks
- The Time E questionnaires were mailed out 2 weeks before the appointment date
- Participant handed in the Time E questionnaire at the appointment, including Maternal and Infant questionnaires and a Breast Feeding Diary;
- Participant might also hand in Paternal E questionnaire if the biological father of the baby was participating;

- Participant completed 24 hour recall, bloodwork, anthropometrics, and provided urine sample, breast milk sample, baby weight, baby samples (e.g. cheek swab, cheek swab and heel poke, or venous draw).

Data management

The data was managed by a data manager (in Edmonton) and the project manager (in Calgary), with consultation of the team biostatistician (co-investigator). Data from questionnaires, excluding nutrition and related data, were entered by various research assistants, and cleaned by the project manager. Nutrition and related data were entered by research assistants and were cleaned by the data manager.

The data base manager (or the designated research assistants) reviewed the completed forms for missing data or for conflicting information. Whenever possible, the data discrepancies were resolved by contacting participants. If the discrepancies were not resolved, they were considered missing data. Once the data had been verified, it was manually entered in either Microsoft Excel or Microsoft Access by data entry staff, who were trained and supervised. Ten percent of questionnaire data from the first cohort of 600 were double entered for accuracy, with over 99% of this data found to be accurately entered. Double entry was employed for the following variables: parity, gravida, gestational age, medical conditions and medications, smoking, alcohol and street drug use, life events, and demographics.

Hard copies of the data were kept secured in locked cabinets in the offices of the lead investigators. Data entered into the computer contained no personal identifiers, and the master files were accessed only by the two data managers. Data request forms for specific variables were sent to the data manager by investigators (and their trainees) in order to access the data for analyses.

Chapter 7, Manuscript 4

***Leung BMY**, Wiens KP, Kaplan BJ. Does prenatal micronutrient supplementation improve children's mental development? A systematic review. BMC Pregnancy and Childbirth 2011, 11:12 (3 February, 2011)*

Given that a high percent of women take supplements during pregnancy (findings in manuscript 3), manuscript 4 examined the global literature to assess the impact of prenatal micronutrient supplementation on the mental development in the children. Research often focused on the effect of supplementation on physical development of the offspring, and less on the impact on mental development. The methods for the systematic review were detailed in the publication and will not be repeated here.

Table 4: List of variables, measurement tools and timeline for data collection in the APrON study

Variable	Variable type	Measurement tool	Time points**
Maternal depression: ➢ Postpartum ➢ Prenatal	1° outcome 2° outcome	EPDS	A, B, C, E
Maternal anxiety	Covariate	SCL-90R subscale	A, B, C, E
Maternal diet	1° predictor	24 hour recall	A, B, C, E
Nutrition status	1° predictor	Blood samples	A, B, C, E
BMI	Covariate	Height and weight	A or B
Social support	Covariate	NPHS Social Support questions – modified	A, B, C, E
Life (stress) events	Covariate	Derived from SLEQ	A or B
Health history	Covariate	Developed for study	A or B
Mental health history	Covariate	Developed for study	A or B
Sociodemographics	Covariate	Derived from NPHS & Community Perinatal Study	A or B

**Time points: A = first trimester (1-13 weeks); B = second trimester (14-26 weeks); C = third trimester (27-42 weeks); E = postpartum (8-12 weeks)

CHAPTER 3: MICRONUTRIENT TREATMENT OF MENTAL DISORDERS

Kaplan B, ***Leung B****. Multi-micronutrient supplementation for the treatment of psychiatric symptoms. Integrative Medicine: A Clinician's Journal, 10(3), Jun-Jul 2011.*

Abstract

Problem: Studying the effect of individual micronutrients on health has been the norm in the research literature for more than a century. It is only recently that a significant shift has been made in traditional medical journals toward accepting the importance of using a complex nutrient formula for intervention research.

Acceptance of this shift is not trivial, as conventional scientific methodology previously branded such interventions as "confounded" experiments. But of course broad-spectrum supplementation is based on fundamental laws of physiology, and important treatment benefits are now emerging in the area of mental health as a result of this shift in attitude.

Review: The focus of this review is the growing body of literature on a broad-based micronutrient formula showing beneficial effects, particularly for mood and anxiety symptoms.

Results: Positive results have been reported in 13 publications on treatment thus far, having been conducted in multiple settings and with many types of designs and analyses, including case reports, case studies, case series in both research and clinical settings, and a retrospective database analysis.

Conclusions: In addition to the review of these studies, 7 conceptual models are presented to help interpret the mechanism(s) by which micronutrients might influence mental health function. Without some understanding of the biological basis for micronutrient interventions for mental health, the benefits tend to be dismissed as being mysterious, when, in fact, they likely reflect fundamental physiological principles of brain function.

Introduction

Hippocrates is credited with the saying "Let food be thy medicine, and medicine be thy food" (121). If indeed Hippocrates made this statement, he was probably referring to using food to nurture both physical and mental health. Although we have no way of confirming that the treatment of mental problems with nutrition was an approach followed by Hippocrates and his colleagues more than 2000 years ago, we do have evidence that the practice was commonplace in North America more than a century ago. At that time, when few North Americans had access to professional health care providers, the *People's Home Library* was found in many homes and farms. It provided handy information about everything from recipes to care of livestock to treatment of tuberculosis or heart disease (122). It is noteworthy that this practical source of information stated that mental illness was known to be the result of "imperfect nutrition" (p. 209).

Standing where we are today and looking back, one could ask the question, "What happened in the ensuing 100 years?*"* One change that is well known is the development of psychiatric medications, especially beginning in the 1950s, for treatment of "mental illness." Less well known, however, is the fact that throughout most of the 20th century empirical studies of nutritional interventions were, in fact, published by many scientific journals. Although seldom referenced in current publications, many medical journal articles from the 1920s onward showed symptom improvement as a result of interventions with nutrients.

In the late 20th century, there were case studies (123) and controlled trials (124) showing amelioration of psychiatric symptoms with some of the B vitamins. Similarly, at the turn of the 21st century, there have been case series (125) and controlled trials (126) demonstrating improvement in mood stability with dietary minerals such as magnesium. Some studies and case

reports of manganese (127, 128) seemed to be especially promising in the 1920s. It is important to note, however, that most interventions for mental disorders consisted of a single nutrient until researcher IR Bell's work in 1992 (129). In that investigation, a combination of vitamins B1, B2, and B6 was found to augment the therapeutic impact of tricyclic antidepressants in 14 seniors suffering from depression.

The Change to Multinutrient Formulas

The year 2000 represented a major watershed in the medical literature on nutrient treatment. Since then, scientific studies employing complex interventions consisting of multiple ingredients have increased significantly. The formulas have varied, containing anywhere from 6 to 36 nutrients. Although this multinutrient approach was a new step for conventional medical journals to report, various complementary, integrative, and orthomolecular journals had, of course, included reports of broad-spectrum interventions for a long time. Integrative-medicine clinicians have always tailored their treatments to the individual patient, using a variety of micronutrients, herbs, and so on.

Designing studies with multiple micronutrients in a single intervention required a change in perspective for academic scientists. The scientific method, which guides most modern scientific research, requires that only a single independent variable be altered in any given investigation. Studies with multiple independent variables are dismissed as containing confounds, preventing proper interpretation of an intervention (130).

This dogma must seem strange to integrative healers, who aim to balance the components of their interventions. Importantly, this dogma is even more incompatible with the best nutrition knowledge that exists. As Burford-Mason expressed so succinctly, "[S]tudying the health effects of vitamins one at a time, or even in small combinations, breaks [a] fundamental law of

physiology" (130). Even so, perhaps it is not surprising that reports of multi-ingredient micronutrient formulas in journals that are typically devoted to allopathic medicine are frequently less than a decade old. Being open to studying a multi-nutrient approach represents a fundamental shift in scientific methodology.

Multiple Micronutrients for Treatment of Mental Disorders

There is now evidence that, in many areas of mental health, the most promising empirical reports have evaluated broad-spectrum micronutrient formulas. These mental health areas include obsessive-compulsive disorder (OCD) (131, 132), mood and anxiety disorders (131-140), attention-deficit hyperactivity disorder (ADHD) (140-142), mood and irritability symptoms associated with autism (143), as well as aggressive or antisocial behaviour in samples not clinically referred, such as in schoolchildren (144) and young offenders (145). There is even 1 excellent controlled trial that used a sample of 300 adults who were basically healthy but who scored relatively high on a measure of life stress (146); those receiving a formula of 10 micronutrients subsequently reported less stress and anxiety than those receiving a placebo.

Complementary to this topic is extensive work on early brain development and cognitive function (147), which will not be included here. Additionally, the omega-3 fatty acid literature—equally important to mental health is also too vast to consider here. Our ensuing comments are restricted to multi-micronutrient interventions for the treatment of mental health problems.

Although other formulas are being studied, the largest body of literature we found is on a single multi-micronutrient supplement that employs a 36-ingredient formula currently called EMPowerplus.[a]

[a]Developed by Truehope Nutritional Support Ltd, the formula was initially called EMPower. In 2002, the company changed manufacturers to use methods that decreased the number of capsules and improved the bioavailability of the product. The resulting product has the same 36 ingredients, but was given the new name of EMPowerplus. The current formula can be found on the developer's Web site (http://truehope.com/default.aspx). It consists of all 14 of the known vitamins, 16 dietary minerals, 3 amino acids, and 3 antioxidants.

Other formulas have many similarities; eg, the one used by Gesch and colleagues (145) consisted of 26 micronutrients. In that study, 172 young-adult prisoners with histories of antisocial and violent behaviour were randomly assigned to receive a placebo or a micronutrient formula with 25 vitamins and minerals plus omega-3 fatty acids. Those receiving the placebo exhibited a 6.7% decrease in disciplinary incidents, but those in the active treatment arm showed a decrease of 35.1%.

Similar results had been reported previously in a randomized, controlled trial involving 80 school children assigned to receive a placebo or a formula with 23 micronutrients. Rule infractions were significantly reduced in those in the active treatment arm (144). Our own work has focused on EMPowerplus, the formula common to the studies that we review here. See Table 5 for a complete ingredient listing.

Research on this multi-micronutrient formula is currently being conducted by academic researchers and physicians in 3 different countries. So far, these investigations have resulted in 14 publications, 13 of which are on efficacy (11 were peer-reviewed, 1 was a commentary, and 1 was a letter to the editor) (131-143). As of this writing, several other papers are in preparation. In addition, a paper on its safety and tolerability in both adults and children has now been published (148). Table 6 describes the 14 that are published and in press. One of the strengths of this literature is that positive results have been reported with many types of designs and analyses, including case reports (with historical information going back 2 to 6 years), case studies (some with within-subject crossover designs), case series (some showing large effect sizes in small samples of children and adults), clinical case series from 2 psychiatrists, and two retrospective

database analyses of 358 adults and 120 children and adolescents. Studies in progress include additional case studies; case series; and a randomized, placebo-controlled trial.

An overview of the results of the 13 treatment studies published thus far should also mention the variety of symptoms that have been targeted and have been shown to be ameliorated by the EMPowerplus formula. Initially, in the publications from 2001 to 2004, the focus was on unstable mood (bipolar symptoms, explosive rage); more recently there have been reports of reduced anxiety and improved OCD symptoms, as well as elimination of symptoms of psychosis such as hallucinations, with use of the formula. One area that is just beginning to be evaluated is ADHD. As recently reviewed (149), of all the nonpharmaceutical treatments proposed and evaluated for ADHD, the multiingredient micronutrient formulas are the most promising—but much more research is required for adequate interpretation.

The studies on EMPowerplus have provided some convincing support for using this formula to treat a number of mental health disorders. The strengths of these studies have included the level of detail on patient history including psychiatric diagnosis and symptoms, medication use (number and types), length of follow-up observations, and the use of standardized psychological instruments.

Since no placebo-controlled trial has yet been published, perhaps it is also worthy of note that both the published database analyses (134) included tracking patient data for 6 months (133, 134), much longer than the typical drug trial in psychiatry (which usually lasts 6 to 8 weeks), and longer than any placebo effect is likely to last. In summary, there are important strengths in this small body of literature about micronutrient mental health treatment that should be acknowledged; positive results for the treatment of mood stability and anxiety symptoms are

consistent across a) several countries, b) multiple universities' investigators, c) different clinic settings, d) all age groups, and e) a variety of research designs.

However, there are also limitations that need to be addressed in future studies. One limitation is bias, particularly observer bias and self-selection bias. Observer bias can influence outcome because the observer (generally clinician, parent, or patient) has knowledge of the intervention and expectations. One way to control for this type of bias is to blind all participants to the intervention. In the case of a within-subjects crossover design, use of a placebo during the withdrawal phase—where clinician, parent, and patient are blinded to the intervention (active versus placebo)—would remove bias in reporting the outcomes. This method is being used in at least 2 other studies currently underway, but has not been used in any of the published reports.

Controlling for self-selection bias, where patients volunteer (or self-select) to take the formula and thus may have expectations of positive outcomes, requires random allocation of patients to a treatment versus placebo or comparator. This method is being used in 1 other study currently underway, but, again, has not been used in any of the published reports. Other limitations of the studies reported thus far are lack of comparison groups (although 1 case-control study is currently under review), and absence of assessment of compliance to ensure that the capsules are taken as prescribed. However, these limitations do not mitigate the positive outcomes experienced by patients in the studies reported thus far.

Biologic Rationale

As we have discussed elsewhere (4), without some understanding of the biological basis for micronutrient interventions for mental health, it is too easy to dismiss the benefit as being mysterious or, worse, a placebo effect (although we note that emerging literature demonstrating

remarkable changes in brain function as a result of "placebo" intervention may cause us all to reconsider this terminology (150).

It is hardly necessary to remind this audience that the biologic functions of vitamins and minerals have been well defined in human, animal, and cellular studies over many decades. Zinc is often mentioned as an illustration of the detailed information available, as it has been shown to be important in more than 200 enzymatic reactions influencing virtually all aspects of metabolism (151). Hence, it is obvious that micronutrient supplementation is likely to influence brain function. The question remains, however, "Influence it via which mechanism(s)?"

In a previous article (4), we suggested 4 possible mechanisms by which micronutrients could influence mental function. These 4 mechanisms are not mutually exclusive but may intertwine and overlap, and they certainly are not exhaustive. Therefore, we append to these 4 previously described explanatory models several other possibilities.

Inborn errors of metabolism: As Ames has shown (152) at least a third of all currently known genetic mutations diminish binding affinity for a coenzyme by a known enzyme. Hence, suboptimal levels of a coenzyme (many of which include micronutrients) would result in decreased binding affinity and a lower rate of metabolic activity. Ames and colleagues reviewed 50 human genetic diseases associated with this type of genetic defect (153). Interestingly, in most cases, the symptoms were treatable by giving the patient additional cofactors—ie, micronutrient therapy. In other words, it is possible that some mental illness, for which genetic predisposition is well-established, is due to inborn errors of metabolism that slow metabolic activity involving neurotransmitters in the brain. Micronutrient treatment might facilitate that metabolic activity, perhaps analogous to the way serotonin-reuptake inhibitors facilitate it by enhancing the supply of this important neurotransmitter.

Deficient methylation: Methylation (adding a methyl group [CH3] to a molecule) is vitally important to proper biologic function. It switches on genes, activates enzymes, and regulates the amount of protein generated by genes. Both DNA transcription and neurotransmitter synthesis are dependent on methylation processes. A methyl donor that is probably familiar to the reader is S-adenosyl-l-methionine (SAM), which is sometimes used in the treatment of depression. The relevance in the current context is that micronutrients are central to adequate methylation activity. Perhaps micronutrient treatment enhances methylation of important brain enzymes, resulting in enhanced neurotransmitter synthesis.

Alteration of gene expression: This model is, in some ways, identical to the deficient methylation just mentioned, though examined in the reverse, as suboptimal nutrient levels can lead to deficient methylation processes. It cannot hurt to reiterate this issue from various perspectives when discussing mental health, as there appears to be strong genetic loading for virtually all mental disorders (154). On the other hand, as with most genetic predispositions, the genetic effects are not deterministic. It is interesting to consider that the expression of a genetic predisposition for a mental disorder may rest significantly on the level of nutrition in an individual. This possibility is strongly supported by the research emerging from the fields of nutrigenomics and epigenetics, showing that nutrient status is a strong modifier of genetic expression.

Long latency effects and the triage theory: One of the most important theories on the topic of micronutrient action was proposed recently by Ames(155) as the triage hypothesis, which posits that nature ensures our survival by rebalancing our metabolism when the availability of the approximately 40 identified micronutrients (vitamins, minerals, amino acids, and fatty acids) is inadequate. What nature does is direct any available micronutrients toward the

processes that are essential for immediate survival, even at the expense of long-term health. Consequently, long-term degenerative disorders such as cancer and other diseases of aging may reflect the cumulative effects of suboptimal levels of micronutrients.

In physical health, this phenomenon is accepted in certain circumstances; for instance, osteoporosis as a manifestation of years of insufficient calcium absorption. But this phenomenon may also be relevant to mental health, as many individuals do not experience their first episode of mental illness until after decades of life, suggesting that the underlying dysfunction develops with age. Perhaps mental disorders reflect the cumulative effects of the body triaging suboptimal levels of micronutrients to metabolic functions critical for survival, at the expense of those brain-related functions necessary for good mental function.

Down-regulation of micronutrient receptors: Readers of this journal are likely familiar with the lifelong commitment to investigating the role of niacin in mental health by the late Abram Hoffer, PhD, MD (156). Very recently, the work of Miller and her colleagues provided a detailed understanding of niacin-receptor activity (157) that they believe could explain observations made by Hoffer and others. Miller et al showed that postmortem brain tissue from 12 patients with schizophrenia was significantly deficient in a particular protein critical for a niacin receptor, even when compared to tissue from 14 patients with bipolar disorder, and certainly when compared to 14 controls.

They suggested that their results were consistent with the blunted niacin-flush response that Hoffer and others had observed for years in people with schizophrenia. Though this particular study is specific to niacin, perhaps researchers in future years will unmask analogous processes related to other micronutrients. Perhaps impaired brain activity in the mentally ill is

sometimes due to inherited deficiencies in protein regulation of micronutrient receptors, which would be improved by micronutrient supplementation.

Impaired growth and development of neurons: There is a remarkably large and growing body of literature showing reduced brain tissue in association with poor mental health. Glial density and neuronal size have been found to be reduced by more than 20% in the anterior cingulate cortex of people with depression (158), brain volume is reduced in people suffering from schizophrenia from a young age (159, 160), and gray-matter deficits are especially well documented in left-frontal regions of people with bipolar disorder (161).

From the perspective of nutrient deficiencies, it may be especially important to emphasize that some of these studies on cortical thinning have demonstrated that the changes are progressive (159). For instance, proton magnetic-resonance spectroscopic evidence has shown progressive changes in the right hippocampus in 15 patients with familial bipolar I disorder (162), with a very high negative correlation (-0.66) between years of illness and reduced N-acetyl-aspartate concentrations. These results suggest that with bipolar disease the brain becomes gradually less able to produce this amino acid, which is ordinarily the second most plentiful in human brain (glutamate being the most abundant).

It is also noteworthy that cortical thinning has been shown to be present in asymptomatic individuals who are at increased familial risk for mood disorders (163). Perhaps the long-term experience of suboptimal nutrition, especially in an individual with a genetic predisposition, results in diminished capacity to maintain and initiate neuronal growth and development, producing vulnerability to mental illness.

Impaired mitochondrial function: There is a growing body of literature demonstrating mitochondrial dysfunction to be the underlying pathophysiology in a variety of neurological and

psychiatric disorders (164-166). In studies too numerous to review here, evidence demonstrates that elevated levels of oxidative stress are associated with neuronal damage in patients and in animal models of brain dysfunction (167). Not surprisingly, micronutrients are the primary treatment for mitochondrial disorders, in addition to carnitine (derived from amino acids). Perhaps psychiatric disorders are expressions of cumulative deficiencies of micronutrients needed to deal with oxidative stress.

We hope it is clear to the reader that many of these 7 mechanisms could interrelate. For instance, alteration of gene expression through inadequate resources for methylation may be influenced by the triage theory so that long-term health is sacrificed for immediate survival needs.

Discussion and Summary

To appreciate the importance of multi-micronutrient research requires a shift in thinking regarding the scientific method, because to be faithful to fundamental laws of human physiology, the independent variable must consist of more than 1 nutrient in any given intervention trial. The bias in traditional medical journals against this way of thinking is beginning to wane, and so an increasing number of articles are being published showing treatment effects of broad-spectrum formulas. We reviewed the growing world literature on one such formula, called EMPowerplus, for which there are promising reports for a variety of mental health problems, especially mood instability and anxiety.

Given the current Western diet, the likelihood of suboptimal nutrition affecting our population seems to be high. In a recent article in which we reviewed the nutritional link to perinatal mood (168), we pointed out the wealth of research demonstrating suboptimal nutrition during pregnancy, the time in life when optimal nutrition is critical. Pregnant women usually

take nutrient supplements and are exposed to an unusual amount of educational information on healthy eating, which in theory should protect this period of gestation from nutrient deficiencies. However, as one example, a study in the United States found mean intake for energy (in calories, iron, zinc, calcium, magnesium, folate, and vitamins D and E from food to be below recommended standards in pregnant adolescents and adults (169).

It is difficult to do research on multi-micronutrient interventions for several reasons; residual bias against complex interventions presents obstacles to obtaining research funds, to getting community support and referrals for studies, and to publishing the results. In spite of these obstacles, we appear to be in a watershed decade, with an increasing number of studies being published that involve broad-spectrum formulas. In this context, it is refreshing to see how important contributions can be from people who think of themselves solely as clinicians and not as scientists. A number of the designs mentioned here, such as case series and within-subject crossover trials, can be employed by clinicians to provide evidence on using a multi-nutrient formula for mental health disorders, especially if standardized tools and systematic data-collection methods are used. As others have said, we need more practice-based medicine, as well as evidence-based practice.

In 1920 the Western world knew and accepted that “imperfect” nutrition was key to mental illness, and the treatment of choice was to improve food intake. Now, almost a century later, research is confirming that prior knowledge, although “suboptimal” nutrition is the term of choice, and treatment usually consists of supplementation in addition to food intake. What remains constant in this century of change is the understanding by some that supplementation is more likely to be effective if provided in the form of broad-spectrum formulas.

Table 5:. Ingredients for 4 EMPowerplus Capsules

Vitamin A (retinyl palmitate)	1536 IU
Vitamin C (ascorbic acid)	160 mg
Vitamin D (cholecalciferol)	384 IU
Vitamin E (d-alpha tocopheryl succinate)	96 IU
Vitamin B1 (thiamine mononitrate)	4.8 mg
Vitamin B2 (riboflavin)	3.6 mg
Vitamin B3 (niacinamide)	24 mg
Vitamin B5 (d-calcium pantothenate)	5.8 mg
Vitamin B6 (pyridoxine hydrochloride)	9.6 mg
Vitamin B9 (folic acid)	384 mcg
Vitamin B12 (cyanocobalamin)	240 mcg
Vitamin H (biotin)	288 mcg
Calcium	352 mg
Phosphorous	224 mg
Magnesium	160 mg
Potassium	64 mg
Iodine (from Pacific kelp)	54.4 mcg
Zinc	12.8 mg
Selenium	54.4 mcg
Copper	1.9 mg
Manganese	2.6 mg
Chromium	166.4 mcg
Molybdenum	38.4 mcg
Iron	3.7 mg
Proprietary Blend	444.1 mg
dl-phenylalanine, glutamine, citrus bioflavonoids, grape seed extract, choline bitartrate, inositol, ginkgo biloba, methionine, germanium sesquioxide, boron, vanadium, nickel	
Other Ingredients: gelatin, silicon, magnesium stearate	

Table 6: Summary of Publications on EMPowerplus to Date

Note: Most adults consume five capsules three times a day, for a total daily dose of 15 capsules; most children consume four capsules three times a day, for a total daily dose of 12 capsules.

Ref	Design	Measurement	Outcome	Strengths	Limitations
(131)	Within-subject crossover trial, 18-year-old male with OCD, Asperger's Syndrome	Mental and behavioral: BDI, BAI, GAF, CBCL, OQ, Y-BOCS	One year of prior experience with CBT as baseline. After 8 wks active treatment, BDI relatively high, but CBCL, BAI, and Y-BOCS low, and OQ score within range found in community samples. In the post-treatment withdrawal period, mild symptoms returned after 10 days; after 8 wks increased severity of obsession and anxiety, decreased mood. Twelve days after reintroduction of formula, improved scores on BDI, BAI, and Y-BOCS; by wk 4, OCD symptoms in remission on Y-BOCS.	Baseline measures permitted follow-up comparison; detailed history of diagnosis, medication use, and other forms of intervention (e.g., CBT); ability to compare patient's responses to medications, CBT, and micronutrients	Unblinded
(132)	Two case	Mental and	8-year-old: Baseline, then treatment for 16	Two-year follow-up mitigated the	Unblinded

Ref	Design	Measurement	Outcome	Strengths	Limitations
	studies, within-subject crossover design: one boy aged 8 (with atypical OCD) and one boy aged 12 (with pervasive developmental delay and explosive rage)	behavioral: Conners PRS, CBCL for both; Y-BOCS for the boy with OCD	wks. At end of treatment, PRS, CBCL, and Y-BOCS scores significantly ↓. At wk 3 of withdrawal of treatment, return of obsessive thoughts; by week 6, increase in CBCL, PRS, and Y-BOCS scores. After 11 wks back on treatment , Y-BOCS was lower than previous period of withdrawal; by wk 18, behavior improvement sufficient for reintegration into regular school. 12-year-old: Baseline, then treatment for 15 wks. After 3 wks treatment, PRS score ↓; continued improvement to wk 15; 3 wks following withdrawal, symptoms returned and PRS scores returned back to baseline levels. Reintroduction of formula led to ↓ CBCL and PRS scores.	effects of positive expectation bias, which likely would have habituated; unplanned crossover sequence allowed comparison of active treatment and withdrawal periods.	

Ref	Design	Measurement	Outcome	Strengths	Limitations
(133)	Database analysis, 120 children and adolescents with bipolar disorder	Parent report of symptom severity based on DSM-specified symptoms	Mean symptom severity of bipolar symptoms was 46% lower than baseline (ES = 0.78) ($p <$ 0.001). In terms of responder status, 46% experienced >50% improvement at LOCF, with 38% still taking psychiatric medication (52% drop from baseline) but at much lower levels (74% reduction in number of medications being used from baseline). The results were similar for those with both ADHD and pediatric bipolar disorder: a 43% decline in bipolar symptoms (ES = 0.72) and 40% in ADHD symptoms (ES = 0.62).	Large sample size; 79% taking psychiatric medications supportive of diagnosis; repeated reporting points extending for 6 months	Unblinded, lack of a control group, self-selection bias
(134)	Database analysis, 358 adults with bipolar	Self-report of symptom severity based on 16 DSM-specified	After 3 mos of taking micronutrient formula, mean 41% ↓ of symptom severity; 45% ↓ at 6 mos. Linear-regression analyses over mos 1–6: symptom decreases were associated with ↓	Large sample size; 81% taking psychiatric medications supportive of diagnosis; multiple reporting points over 6 mos	Unblinded, lack of a control group, self-

Ref	Design	Measurement	Outcome	Strengths	Limitations
	disorder	symptoms	meds and ↑ dosage of formula.		selection bias
(135)	Case report, 12-year-old boy with 6 yrs history of bipolar disorder	Parental report as well as psychiatrist and psychologist evaluations	After 6 years psychiatric treatment, patient transitioned to micronutrient formula. In 19 days all symptoms remitted: irritability, headache, dizziness, fatigue, hallucinations, compulsions; also, improvements were noted in attention and sleep.	Six years documented history with traditional treatments, observed by parents and mental health professionals; detailed medication history	Unblinded; absence of standardized data collection
(136)	Case series of 11 children aged 8-15 years with mood and behavior problems	Mental and behavioral: YMRS and YOQ at entry and each follow-up visit; CBCL twice	↓ scores after minimum of 8 wks treatment for 7 of 8 CBCL subscales; YOQ significantly improved post-treatment, as well as YMRS (available for only 4 children).	Significant findings despite small sample size; large effect sizes (>0.80) for each CBCL scale, the YOQ, and the YMRS	Unblinded
(137)	Case series, 14 adults	Mental and behavioral:	After minimum of 6 mos treatment, significant ↓ in scores for the HAM-D (55%↓), YMRS	Large ES (>0.80) for each of the 3 outcome measures despite a small	Unblinded

Ref	Design	Measurement	Outcome	Strengths	Limitations
	aged 19-46; bipolar I, II, and NOS	HAM-D, BPRS, YMRS	(66%↓) and BPRS (60%↓). Patients able to be managed on fewer medications (>50% reduction), from an average of 2.7 $\pm$ 2.0 to 1.0 $\pm$ 1.1.	final sample size of 11 patients	
(138)	Clinical case series, 19 adults with bipolar I (n=14), and II (n=5), followed for 5-21 mos	Clinician evaluation of mild, moderate, or marked improvement	Positive response in 16 patients (12 marked, 3 moderate, 1 mild improvement); 13 completely discontinued psychiatric medications and remained stable on micronutrient formula alone.	Unselected sample, monitored in naturalistic clinical setting	Unblinded
(139)	Clinical case series, 22 patients (10 adults, 9	Clinician evaluation of mild, moderate, or marked	Positive response shown by 19 patients (10 marked, 7 moderate, 2 mild improvement); 11 were stable for 6-9 months post treatment without psychiatric medications.	Unselected sample, monitored in naturalistic clinical setting	Unblinded

Ref	Design	Measurement	Outcome	Strengths	Limitations
	adolescents, 3 children) with bipolar disorder	improvement			
(140)	Case study of of 21-year-old female with bipolar disorder II and ADHD; naturalistic crossover design	Mental and behavioral: CGI-S, CGI-I, MADRS, YMRS, CAARS, plus cognitive tests of memory, learning, etc.	After 8 wks on the formula, significant improvements in mood, anxiety, and hyperactivity/impulsivity; 8 wks after stopping the formula, depression scores returned to baseline, and anxiety and ADHD symptoms worsened. After reintroduction of the formula, gradual improvement in all psychiatric symptoms occurred and were sustained at 12 ms.	Diagnosis confirmed with structured interview; one year follow-up data reported; neurocognitive changes mirrored behavioral changes, showing improved processing speed, consistency in response speed, and verbal memory.	Unblinded
(142)	Matched group comparison	Cognitive tests of memory, learning,	After 8 wks micronutrient treatment, the most salient group difference was improvement only in the ADHD group across a range of verbal	Diagnoses confirmed with structured interview; first demonstration of cognitive	Unblinded

Ref	Design	Measurement	Outcome	Strengths	Limitations
	of 14 adults with ADHD and severe mood dysregulation, and 14 controls, to determine if changes in cognitive function were attributable to practice effects	attention, executive function	abilities including verbal learning, verbal cognitive flexibility and fluency, and verbal inhibition	improvements following micronutrient treatment; control group ruled out practice effects as explanation	
(141)	8-wk open label trial	Mental and behavioral: CGI-	Significant improvements on measures of inattention and hyperactivity/impulsivity,	Diagnosis confirmed with structured interview; unselected	Unblinded

Ref	Design	Measurement	Outcome	Strengths	Limitations
	with 14 medication-free adults (9 men, 5 women; 18-55 years) with ADHD and severe mood dysregulation	S, CGI-I, MADRS, YMRS, CAARS	mood, quality of life, anxiety, and stress, all with medium to very large effect sizes (all Ps < .01); follow-up data showed maintenance of changes or further improvement for those who stayed on the micronutrients.	sample of a very difficult-to-treat combination of symptoms (ADHD and severe mood dysregulation); multiple observers (self, observer, clinician); significant improvement in quality of life; natural extension to compare those who continued vs stopped taking the formula	
(143)	A naturalistic case-control study comparing micronutrient (n=44) to	Mental and behavioral: CARS, CPRS, ABC, CGI-S	Both groups improved on both the CARS and CPRS (all p values <0.0001). Both groups also exhibited significant decreases in total ABC scores, but the micronutrient group's improvement was significantly greater (p<0.0001). Self-injurious behavior intensity	Comparison groups, matched for age, gender, and symptom severity	Unblinded; self selection bias

Ref	Design	Measurement	Outcome	Strengths	Limitations
	medication (n=44) treatment in people on the autism spectrum		was lower in the micronutrient group at the end of the study (p=0.005), and improvement on the CGI-S was greater for the micronutrient group (p=0.0029).		
(148)	Biological safety data from 144 children and adults from six sources; Tolerability data (Adverse Events) available	Hematology, clinical chemistry, urinalyses	Safety results: No clinically meaningful negative outcomes/effects or abnormal blood tests that could be attributed to toxicity of the formula. Tolerability results: only minor, transitory reports of headache and nausea.	Multiple samples for comparison, from multiple laboratories and investigators	Only one direct comparison of formula to medication (reference 23 above)

Ref	Design	Measurement	Outcome	Strengths	Limitations
	from 157 children and adults from six studies				

Key: **ABC**=Aberrant Behavior Checklist, **ADHD**=attention-deficit hyperactivity disorder, **BAI**=Beck Anxiety Inventory, **BDI**=Beck Depression Inventory, **BPRS**=Brief Psychiatric Rating Scale, **CAARS**=Conners Adult ADHD Rating Scales, **CARS**=Childhood Autism Rating Scale, **CBCL**=Child Behavior Checklist, **CBT**= cognitive behavior therapy, **CGI-S** and **CGI-I**=Clinical Global Impressions Severity and Improvement Scales, **CPRS**=Childhood Psychiatric Rating Scale; **DSM**=Diagnostic and Statistical Manual of the American Psychiatric Association, **ES**=Effect Size, **GAF**=Global Assessment of Functioning, **HAM-D**=Hamilton Rating Scale for Depression, **LOCF=**last observation carried forward, **MADRS**=Montgomery-Asberg Depression Rating Scale, **mo**=month, **NOS**=not otherwise specified, **OCD**=obsessive compulsive disorder, **OQ**=Outcome Questionnaire, **PRS**=Conners Parent Rating Scale, **wk**=week, **Y-BOCS**=Yale-Brown Obsessive Compulsive Scale, **YMRS**=Young Mania Rating Scale, **YOQ**=Youth Outcome Questionnaire

CHAPTER 4: PERINATAL DEPRESSION: PREVALENCE, RISKS AND THE NUTRITION LINK – A REVIEW OF THE LITERATURE

***Leung BM**, Kaplan, B. Perinatal Depression: prevalence, risks and nutrition – a review of the literature; Journal of the American Dietetics Association, 2009. 109: 1566-1575.*

Abstract

The purpose of this review is to examine the role of nutrition in perinatal depression. Perinatal (maternal) depression refers to major and minor episodes during pregnancy (termed antenatal) and/or within the first 12 months after delivery (termed postpartum or postnatal). The prevalence of antenatal depression may be as high as 20%, while approximately 12 - 16 % of women experience postpartum depression. These are probably conservative estimates as cases of maternal depression are under-reported or under-diagnosed. The risk factors for depression include genetic predisposition and environmental factors, as well as a number of social, psychological and biological factors. One biological factor given increasing consideration is inadequate nutrition. Credible links between nutrient deficiency and mood have been reported for folate, vitamin B12, calcium, iron, selenium, zinc and omega-3 fatty acids. For maternal depression, the nutrient that has received the most attention from nutrition researchers has been the omega-3 essential fatty acids. Numerous studies, such as randomized controlled trials, cohort studies, and ecological studies, have found a positive association between low omega-3 levels and a higher incidence of maternal depression. In addition, nutrient inadequacies in pregnant women who consume a typical Western diet may be much more common than researchers and clinicians realize. A number of studies have reported inadequate intakes of omega-3, folate, B vitamins, iron, and calcium in pregnant women. Depletion of nutrient reserves throughout pregnancy may increase a woman's risk for maternal depression.

Introduction

There is growing concern over the rising prevalence of mental illness in the world (170). One of the major mental health problems has been mood disorders, the most common of which worldwide is depression. According to the World Health Organization (WHO), depression was the leading cause of disability worldwide and the fourth leading contributor to the global burden of disease (the disability adjusted lives per years or DALYs) (6). By the year 2020, WHO predicts that depression will be the second largest contributor to the global DALYs for all ages and both sexes (6). Today, depression is already the second largest cause of DALYs for those of reproductive age (15 to 44 years of age). Women are two to three times more likely to experience depression than men (19). The consequences of depression in women impart greater significance during or after pregnancy. While depression in general can have devastating effects on families and the community, maternal depression has much more serious and long lasting consequences for the children in the family, as will be shown below.

There are a number of theories as to the causes and risk factors for depression. This article will examine the link between food and mood, one possible cause of depression that is of growing interest in the scientific community (60, 62, 63, 171). Findings presented in this article will demonstrate that diets with various nutrient deficits are more common than recognized (169, 172), and that such deficits may contribute to maternal depression.

The purpose of this review is to examine the prevalence of depression in women during pregnancy and the postpartum period, and then to assess whether nutrition may play a role. The literature on of the relationship between food and mood raises the question of whether nutrient deficiency might be a significant contributor to the development of depression in women during and after pregnancy.

Perinatal Depression

Perinatal depression refers to major and minor episodes during pregnancy (termed antenatal) or within the first 12 months after delivery (termed postpartum or postnatal) (11). The term "maternal depression" has also been used interchangeably with perinatal depression. The signs and symptoms for perinatal depression are the same as those for depression in the general population: depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration (12).

To date, there are no diagnostic criteria for perinatal depression *per se*; diagnosis is based on the Diagnostic and Statistical Manual, fourth edition or the International Statistical Classification of Diseases, tenth edition (12). The prevalence of antenatal depression appears to peak in the first trimester, while postpartum depression peaks around twelve weeks post-delivery (11). Both scientific research and public awareness have focused primarily on postpartum depression; however, it is now recognized that antenatal depression is just as problematic (perhaps more) as postpartum depression and the two may be a continuum reflecting an underlying chronic condition among women during pregnancy and thereafter.

Antenatal Depression (AD)

Antenatal depression (AD) is a significant health problem, but is less well-studied than postpartum depression (12). It has been estimated that the prevalence of AD may be as high as 20% (173). For example, a cross-sectional study of 432 women seen in a private Brazilian clinic used the Beck Depression Inventory as a screening tool and reported a prevalence of 19.6% for AD (174). Previously, in a larger sample of over 3000 pregnant women in the U.S., 20% scored above the cutoff for depression on the Center for Epidemiological Studies Depression scale (175). Both measures used in these studies are well-validated for self-report of mood and the

similarity of prevalence rates is striking. A systematic review by Bennett and colleagues found prevalence rates at 7.4%, 12.8% and 12.0% for first, second and third trimesters respectively (176). These are likely to be conservative estimates as women who are depressed are less likely to participate in research studies.

Postpartum depression (PPD)

Postpartum depression (PPD) has received more attention in the medical and scientific literature than AD. Published estimates indicate that approximately 12 - 16 % of women experience PPD (177). Like AD, this is probably a conservative estimate as cases of PPD are under-reported or under-diagnosed. In fact, a review by Gavin and colleagues estimated that as many as 19% of new mothers may suffer from depression within the first three months of giving birth (11). PPD emerges most often within 6 to 12 weeks of delivery, but can occur any time up to one year following birth (177). Longitudinal studies have found that depression in the postpartum period can last for months or even years after giving birth (178, 179).

Effects of maternal depression

Maternal depression is a serious mental health problem that can negatively affect the lives of women, children and their families (63, 111, 180). The impact of PPD and AD must be considered both in terms of the women's own lives, as well as their children's. The deleterious effects of maternal depression on a child's functioning in cognitive, social and developmental areas are well documented in the literature (111, 181, 182). For the woman, depression during pregnancy has been linked to poor maternal self care and outcome (176). Depressed pregnant women are less likely to seek proper medical care during pregnancy and more likely to engage in risk-taking activities such as alcohol or drug abuse (173). Poor obstetric outcomes in women with AD include pre-eclampsia, birth difficulties for the mother and child, and almost seven

times the risk for postpartum depression (183). Maternal depression has also been associated with increased risk of preterm delivery and reduced breastfeeding (173).

For the newborn, maternal depression is strongly associated with lower Apgar scores, failure to thrive, and poor physical and emotional / behaviour development (184, 185). A study of 9244 families in the southwest of England reported developmental delay in babies aged 18 months whose mothers were depressed (186). Another study found maternal depression was associated with poorer growth (underweight and stunting) and greater risk of diarrheal disease in a sample of infants in Pakistan (187). A review of cross-sectional and cohort studies by Stewart reported that maternal depression was associated with poor infant physical growth, infant malnutrition and increased problems such as respiratory and diarrheal illness (184). Furthermore, children of depressed women are also at greater risk for lower scores on developmental scales, exhibit reduced motor tone and activity, and have more behavioural problems (188) such as sleep disturbances and irritability (173). Even more disturbing was a study that found that the offspring at 16 years were almost five times more at-risk for depression when born to women with antenatal depression, compared to adolescents born to women without antenatal depression (189). The role of genetic predisposition may be an influence in such data. Thus, maternal depression has broad implications for maternal and child mental and physical health that may have long lasting social and economic impact (190, 191).

It is important to consider that causal arrows may point in both directions: maternal depression may influence child development, but poor infant outcomes can also affect maternal mood. In the case of PPD, it is possible that infant illness, such as failure to thrive for medical reasons or congenital diseases, may contribute to depression in new mothers (see Figure 3). A newborn with an illness is a significant additional stressor for a family which is already

challenged by the adaptations to the changes in their routine and meeting the needs of the baby. However, this bidirectional causal pathway does not explain the fact that the incidence of poor birth outcomes is low relative to the incidence of postnatal depression. In other words, mothers experience postnatal depression at a much higher rate than the number of babies born with poor birth outcomes. Thus, risk factors other than poor infant outcome are likely to play a larger role in maternal depression.

Risk factors for maternal depression

The symptoms of general depression are variable among patients and, consequently, depression has been viewed not as a single disease, but as a syndrome encompassing a spectrum of mood symptoms with multiple causes and possibly multiple pathophysiologies (24). These multifactorial causes of depression likely involve both genetic and environmental factors (25). The genetic basis of mood disorders has been studied extensively. Craddock and Forty reviewed linkage, genetic, family, and twin studies and found evidence implicating specific genes with regard to depression and schizophrenia (25). However, replication of these studies is needed, in addition to studies on phenotypic relationships and biological mechanisms, to determine degree of genetic causality.

The degree of interplay amongst the different factors is unclear. For example, epidemiological data have shown about 40% to 50% of the risk for depression is linked to genetics (24), but genetic studies on mood disorders have yet to provide conclusive evidence of specific susceptibility genes or their pattern of inheritance (30). The uncertainties are likely due to the complex nature of the disorder and also due to the involvement of multiple genes and gene interactions. Thus, it is likely that genetic predisposition in combination with an array of environmental influences is involved in the development of depression.

Environmental factors associated with depression include stress (e.g., physical, mental and emotional trauma), viral infections, hormonal disorders, chronic diseases (24), drugs such as oral contraceptives, and some medications (e.g., sedatives) (192). However, environmental factors do not act alone; they can increase the risk for depression in those with a genetic susceptibility for the disease.

In addition to genetic predisposition and environmental factors that influence the incidence of depression, a number of social, psychological and biological factors have been associated particularly with risk for maternal depression. Social risk factors include lack of partner or marital difficulties (e.g., divorced) (33), low socio-economic status (e.g., financial insecurity/hardship) (32, 193), poverty, lack of social support (174, 194, 195) or social isolation (196), major life events (197), family violence (e.g., history of abuse) (197), increased life stress, and substance abuse (173). Psychological factors include current depression or anxiety (198), history of depression (193, 198), history of psychiatric illness (196) such as premenstrual dysphoric disorder, and mood symptoms during the third trimester (199). The research into social and psychological factors associated with maternal depression has been extensive.

The set of biological risk factors associated with maternal depression is more difficult to ascertain. Biological factors that contribute to the pathophysiology of maternal depression include hormonal influences (188, 200), neurotransmitter function (201-203), and nutrient deficiencies due to malnutrition or poor diet quality (63).

Pathophysiology of maternal depression

A number of mechanisms have been proposed for the pathophysiology of maternal depression, including the hypothalamic-pituitary-adrenal (HPA) axis and the role of cortisol (41, 42). The findings from studies on cortisol and the HPA axis have been contradictory. For

example, Jolley and colleagues reported that there was no relationship between adrenocorticotropic hormone and cortisol levels in subjects with PPD (50). In contrast, the normal reaction of higher adrenocorticotropic hormone and lower cortisol levels was demonstrated in a control group, suggesting that there was some form of "dysregulation" of the HPA in those with PPD (42). However, Zonana and Gorman reviewed a number of studies that showed no association between cortisol levels and PPD (41).

Hormones are another mechanism hypothesized to be involved in the pathophysiology of maternal depression; they include progesterone, estradiol and estriol, prolactin, thyroid stimulating hormone and triiodothyronine/thyroxine (41, 204-206). It is well known that hormonal levels change drastically during and after pregnancy. For example, estradiol levels increase 50 times and progesterone levels 10 times by the third trimester, and they normalize within one to two weeks postpartum. However, no association has been found between the changes in hormonal levels and the peak incidence of depression during or after pregnancy (41). Elevated thyroid stimulating hormone has been found to be associated with higher depression scores (207). Another study found higher risk for PPD symptoms in women with lower levels of antenatal total and free thyroxine levels (48). A number of other studies have examined the role of hormones on PPD; a detailed review is available elsewhere (41). In summary, however, the role of specific hormones remains uncertain.

A majority of the research on the pathophysiology of depression has focused on the monoamine neurotransmitters (serotonin, norepinephrine and dopamine) (201-203). A review by Nemeroff reported decreased or altered levels and activity of the three monoamine systems in the brains of patients with depression (208). Previous research has examined serotonin as the main neurotransmitter responsible for depression. However, in light of the evidence on the lack of

efficacy of anti-depressants such as selective serotonin reuptake inhibitors and selective serotonin-norepinephrine reuptake inhibitors, studies into the dopaminergic system (responsible for pleasure) have increased (46, 49). In other words, it is unlikely that a single neurotransmitter system is responsible for the symptoms of depression. While there is likely an association between the monoamine systems and maternal depression, the literature on pregnant or postpartum women is limited.

An element common to the various mechanisms described above is nutrition. Nutrition provides the basic elements required for biochemical pathways to ensure proper physical and mental development and function; i.e., nutrients provide the underlying foundation for proper function of the HPA axis, other endocrine systems, and neurotransmitter pathways. The general role of nutrition in depression has recently become a focus of investigations. This evidence is best reviewed in the broader context of the role of nutrient intake in relation to mood.

Nutrition and mood

The research on the relationship between nutrition and brain function is remarkably large, and reaches back almost 90 years (4, 209, 210). Credible links between nutrition and mood have been reported for folate (211), vitamin B12 (63, 212), calcium (212, 213), iron (63, 212, 214), selenium (63, 215), zinc (63, 216, 217) and polyunsaturated fatty acids (PUFAs) (63, 64, 93, 95, 96, 218-221). A review of correlational and intervention studies by Kaplan and colleagues found potentially beneficial effects of many vitamins (especially B-vitamins, and vitamins C, D and E), minerals (calcium, chromium, iron, magnesium, zinc, selenium), and vitamin-like compounds (choline) on mood symptoms (4, 222).

Individual nutrients have been studied with respect to their role in a number of neural and endocrine pathways, including how their deficiency may contribute to the pathophysiology of

depression. One of the well-studied vitamins is folate, which is required for the biosynthesis of the three monoamine neurotransmitters, serotonin, dopamine and norepinephrine. The active metabolite of folate, 5-methyltetrahydrofolate (5-MTHF, L-methylfolate), is required for the re-methylation of homocysteine in the production of methionine, which is involved in a number of biochemical processes involving the three aforementioned neurotransmitters (78). Thus, a deficiency in folate would impact the production and function of these neurotransmitters. Another vitamin involved in neurotransmitter pathways is vitamin B6, which is a cofactor in the production of serotonin from tryptophan. Low plasma levels of the B6 derivative, pyridoxal phosphate, have been associated with symptoms of depression (223). Vitamin B12 is another nutrient that is crucial to neurological function. B12 is a cofactor in the formation of S-adenosylmethionine, an intermediate for the production of the neurotransmitters (224). There is evidence from research on laboratory animals that vitamin D, in the form of 1α,25 dihydroxyvitamin D_3, may be involved in anterior pituitary lobe function, as well as in dopamine concentration in the cortex (225). In humans, a study by Lansdowne and Provost found vitamin D to enhance mood in seasonal affective disorder (226). Another nutrient linked to mood is zinc. Studies have reported an association between low zinc status and depression; there is also evidence that intervention with zinc has an antidepressant effect (216, 227). It has been suggested that zinc may influence serotonin uptake (227). Researchers are just beginning to examine individual nutrients and their role in biosynthesis, metabolism and function of various hormones and neurotransmitters. Much more work is needed to understand their roles. The physiological mechanisms of some nutrients and their effects on brain/neurological function are summarized in Table 7.

Nutrition and maternal mood

While specific nutrients associated with depression in the general population have been demonstrated, little is known about low nutrient levels and maternal depression. Most studies on specific nutrients (e.g., vitamins and minerals) and mood have excluded pregnant women. However, it is known that pregnant women are especially susceptible to the effects of low nutrient intakes (63). During pregnancy and lactation, nutritional requirements are increased so that foetal and infant growth are adequately supported, as well as maternal metabolic needs specific to reproduction (228). In other words, the fetus is preprogrammed with specific energy and nutrient needs in order to grow and develop at set times during gestation, and these needs must be met by the mother. Nutrient demands from the fetus change over time and during different developmental stages (229). Thus, it is important that pregnant women select foods with enhanced nutrient density in order to avoid risking nutritional inadequacy, which may have long-lasting effects on both the women and their children (228). Picciano (228) summarized the increased overall nutrient requirements of pregnant women compared to nonpregnant women; however, the nutrient that has received the most attention in relation to maternal depression has been the omega-3 essential fatty acids.

Essential fatty acids and maternal mood

Essential fatty acids are polyunsaturated fatty acids (PUFAs) categorized into two main groups: linoleic acid (omega-6) and alpha-linolenic acid (omega-3). These fatty acids are termed essential as the body does not produce them and they must be obtained through our diet (230). The two omega-3 fatty acids most relevant for brain development and function are eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), of which the latter is the most prominent in the brain. EPA and DHA can be synthesized in the body from alpha-linolenic acid.

Omega-3 fatty acids are essential for receptor function, neurotransmitter uptake and signal transmission. They are also precursors to specific prostaglandins and leukotrienes (chemicals that dilate blood vessels and prevent clotting) (231, 232). The amount of omega-3 in the modern diet has declined with the decreased intake of sources of omega-3 fatty acids (particularly those coming from marine sources). In addition, there has been an increase in the amount of omega-6 fatty acids in the modern diet, which can interfere with the metabolism and synthesis of DHA and EPA (231, 233).

The links between omega-3s (EPA and/or DHA) and perinatal depression have been examined in a number of observational and intervention studies (see Table 2), as well as review papers that have reported the inconsistency among the empirical studies. A review by Freeman found inconsistent results in clinical trials with EPA and/or DHA; some results were better than placebo, while others were not (234). Sample size and duration of follow-up were reported to be problematic in some of the study designs. Another review by Hosli and colleagues found mixed results also; findings from a meta-analysis were inconsistent, and clinical studies contradicted observational studies (235). However, a review of epidemiological evidence and intervention studies by Rees and colleagues reported an association between low omega-3 intake and depression (219). An analysis of ecological studies from 23 countries by Hibbeln revealed that high DHA levels in breast milk and higher seafood consumption were positively predictive of lower rates of postpartum depression (220). De Vriese and colleagues (64) found that omega 3 levels were significantly lower in women who developed PPD than in women who did not. After their critique of study designs and the methodological rigor of multiple case-control studies, cohort studies and double blind randomized trials, Sontrop and Campbell concluded that a relationship between omega-3 PUFAs and depression was biologically plausible (76).

Vitamins/minerals and maternal mood

Outside the PUFA literature, a thorough search of the peer reviewed journal databases found limited research on vitamin/mineral deficiency and maternal depression. Beard and colleagues reported a strong relationship between maternal iron status and depression in a study that followed mothers of full-term normal birth weight babies from 10 weeks to nine months postpartum (236). In another example, results from a study by Wojcik and colleagues showed a relationship between decreased serum zinc concentration and higher scores on the Edinburgh Postpartum Depression Scale (237).

It is recognized that nutrients do not work alone in biochemical pathways that influence mood. A few studies have examined other nutrients in relation to mood disorder in pregnant women with varying results (66, 213). A number of limitations associated with the studies reviewed above (from examining a single nutrient to using a single diet questionnaire) have been recognized. More research into the use of omega-3 fatty acids and other nutrients as potential treatment or prophylactic supplements for depression in women in the antenatal and postnatal periods is needed. The limitations in these studies are similar to those of studies that have examined other specific nutrients and mood, and will be reviewed next.

Limitations of studies

A review of the literature showed that studies have been both correlational (finding, for instance, that depressed patients suffer from poor nutrition) and interventional (demonstrating improved mood following supplementation). However, the evidence on how nutrition affects women's mental health during pregnancy or in the postpartum period is limited. The strengths of some of the studies cited above have been in the identification and correlation of specific nutrients with depression. However, with the exception of studies on omega-3s, most research

that examined the inter-relationships between nutrition and depression has excluded pregnant or lactating women.

Numerous studies were ecological or cross-sectional, measuring nutrition and depression simultaneously, and consequently causal relationships cannot be determined. For example, one of the diagnostic criteria for depression is the experience of altered appetite and body weight, which may in itself result in poor nutrition (181). Randomized controlled trials in this area usually consist of short follow-up periods (e.g., less than 12 weeks), small sample sizes, a single nutrient intervention being studied, and homogeneous samples that do not include pregnant women (63). These findings do not reflect real life practices where nutrients do not function alone in affecting mood and physiology. Thus, multiple nutrients need to be examined concurrently. Furthermore, a number of studies use a single food frequency questionnaire or a single diet recall as estimates of "average" nutrient intake (87, 91). But such point-specific measurements are inadequate in assessing the overall nutrient intake in the sample and may not reflect the nutrient intakes of the population (64, 234).

Previous investigations rarely assessed overall nutrient intake and few studies adjusted for confounders, leading to potentially biased results. For example, a study by Harrison-Hohner and colleagues (213) derived the association between calcium and PPD from ancillary information in a study on calcium and pre-eclampsia. This type of secondary finding can provide information on a possible association between calcium and PPD, but it lacks the credibility of primary evidence of a randomized controlled trial or longitudinal cohort study. While the research has primarily examined the association of nutrition and PPD, no literature is currently available on the influence of maternal nutrition on AD. Thus, longitudinal studies with repeated

measures are needed to determine whether poor nutrient status is associated with depressive symptoms in the antenatal period or the onset of depression in the postpartum period (63).

Dietary inadequacy in pregnant women

The link between nutrient deficiency and maternal depression in developed countries may not seem obvious. However, nutrient deficiencies among those who consume a typical Western diet may be more common than people realize. A study of pregnant adolescents and adults living in the US found mean intake for energy, iron, zinc, calcium, magnesium, folate and vitamins D and E to be below recommended standards in both groups (169). Another study also found pregnant women did not consume adequate amounts to meet the nutrient requirements for calcium, iron, folate (238), omega 3 essential fatty acids (238, 239), and vitamin D (240) (see Table 8). Even middle- to upper-income pregnant women did not consume adequate amounts of iron and possibly magnesium from foods (9). A British study found a high percentage of pregnant women did not meet the estimated average requirement (a nutrient intake value that is estimated to meet the requirement of half of the healthy individuals in a particular life stage and gender group) for calcium (40%), iron (67%), and folate (69%) (241). Machioni and colleagues found inadequate iodine intake in pregnant women even though they lived in a region of Italy reported to have sufficient iodine levels available in their diets (172). A study of obese pregnant women found low status for a number of minerals in both the mother and their fetuses (242). Other studies have also found that pregnant women did not meet daily recommended intakes of nutrients through dietary means (9, 169, 212, 241, 243-245).

Given the evidence of dietary inadequacy in pregnant women and the link between nutrient deficiency and depression, it is reasonable to theorize about the potential influence of nutrient deficiency on the incidence of maternal depression. Proper nutrition during pregnancy is

vital to the health of a woman and her fetus (70, 71), as pregnancy presents unique stresses that challenge overall physical and psychological adaptation in women (246). Women are particularly vulnerable to the adverse effects of poor nutrition on mood because pregnancy and lactation increase the nutrient requirements. It has been proposed by others that depletion of nutrient reserves throughout pregnancy and a lack of recovery postpartum may increase a woman's risk for maternal depression (63).

Conclusion

The literature reviewed here suggests that nutrient intake may be a key factor in a woman's vulnerability to perinatal depression. There is a compelling argument for longitudinal research that targets this important topic as its primary focus: determining whether nutrient status is associated with maternal mental health in pregnant women.

Acknowledgement

Both authors were responsible for interpreting the literature, drafting the manuscript and formulating the final manuscript. The first author (BL) was responsible for literature search and review.

Financial or personal interest disclosure: none.

Table 7: Summary of individual nutrients, mechanism of action and the neurologic consequence of deficiency

Nutrient	Mechanism of Action	Deficiency Effects
Vitamin B1 (thiamine)	Facilitates glucose use for energy production (decarboxylation, transamination, oxidation, reduction reactions) (209)	Neurological changes including confusion, apathy, decreased short term memory and irritability (247); in rat models, selective neuronal cell death in thalamic structures (209)
Vitamin B6	Chemical mediator synthesis; alters N-methyl-D-aspartate receptors in the CNS (248)	Asthenia, irritability, depression (209)
Vitamin B12	Works with folate in methionine-synthase mediated conversion of homocysteine to methionine, which is essential for nucleotide synthesis and methylation (249)	Neurological disorders, psychotic disturbances, hematological alterations; memory loss, pain, abnormal sensations of extremities (249)
Folic acid	Methionine-homocysteine metabolism (209)	Neural tube defects; megaloblastic anemia with affective (mood) disturbances (248, 250)
Vitamin D	Protects neurons of hippocampus, modulates transport of glucose to the brain (251)	Hypothesized role of prenatal hypovitaminosis D on adult neuropsychiatric outcomes (251)

Nutrient	Mechanism of Action	Deficiency Effects
Iodine	Major constituent of thyroid hormones, affects gene expression of other hormones & growth factors (248)	Reduced IQ; cretinism & mental retardation in children born to iodine-deficient moms during pregnancy; hypothyroid-associated depression (248)
Iron	Myelination and lipid metabolism; alters neurotransmission, energy production and DNA synthesis (248)	Reduced learning and memory, behavioral abnormalities (affect & interpersonal interactions) (248); Impaired mood & cognition (236)
Selenium	Component of selenoprotein glutathione peroxidase which plays an important role in the anti-oxidant mechanisms; required for synthesis and metabolism of thyroid hormones (215)	At times of deficiency the brain retains selenium to a greater extent than any other organ; low selenium intake associated with poorer mood (215).
Zinc	DNA and protein synthesis (248)	Impaired learning and response to stimuli, reduced activity and attention (248); impaired body accumulation of PUFAs (209)
Omega 3 fatty acids	Constituent of cell membranes, substrate for lipid-derived	Impaired vision, hearing, olfactory functions; reduced membrane

Nutrient	Mechanism of Action	Deficiency Effects
	mediators for cell-to-cell communication and signal transduction (248)	renewal, thus accelerated cerebral aging. Thought to be associated with mood, depression, dementia; may have role in prevention of aforementioned disorders (171)

Table 8: Summary of studies on nutrient inadequacies in pregnant women

Study	Sample size	Measurement tool	Outcome
Giddens, 2000 (169)	59 pregnant adolescents & 97 pregnant adults	Two 7-day food records	Mean intakes for *energy, iron, zinc, calcium, magnesium, folate and vitamins D & E* below recommended standards in both groups
Turner, 2003 (9)	63 middle- to upper-income women	3-day diet records each month during pregnancy	Foods less than the EAR were *iron, magnesium, zinc, vitamin B6, selenium & vitamin C*
Denomme, 2005 (239)	20 pregnant women	Duplicates of actual foods eaten on 3 separate days (sent to lab for analysis) 3-d dietary record	PUFA (65%) and DHA (90%) levels below Acceptable Macronutrient Distribution Range
Mouratidou, 2006 (241)	250 pregnant women	Interviewer-administered semi-quantified food frequency questionnaire	40 % of participants did not meet the EAR for calcium, 67 % for iron and 69 % for folate.
Sherwood,	61 pregnant	Weighed food	36% of pregnant women and 32%

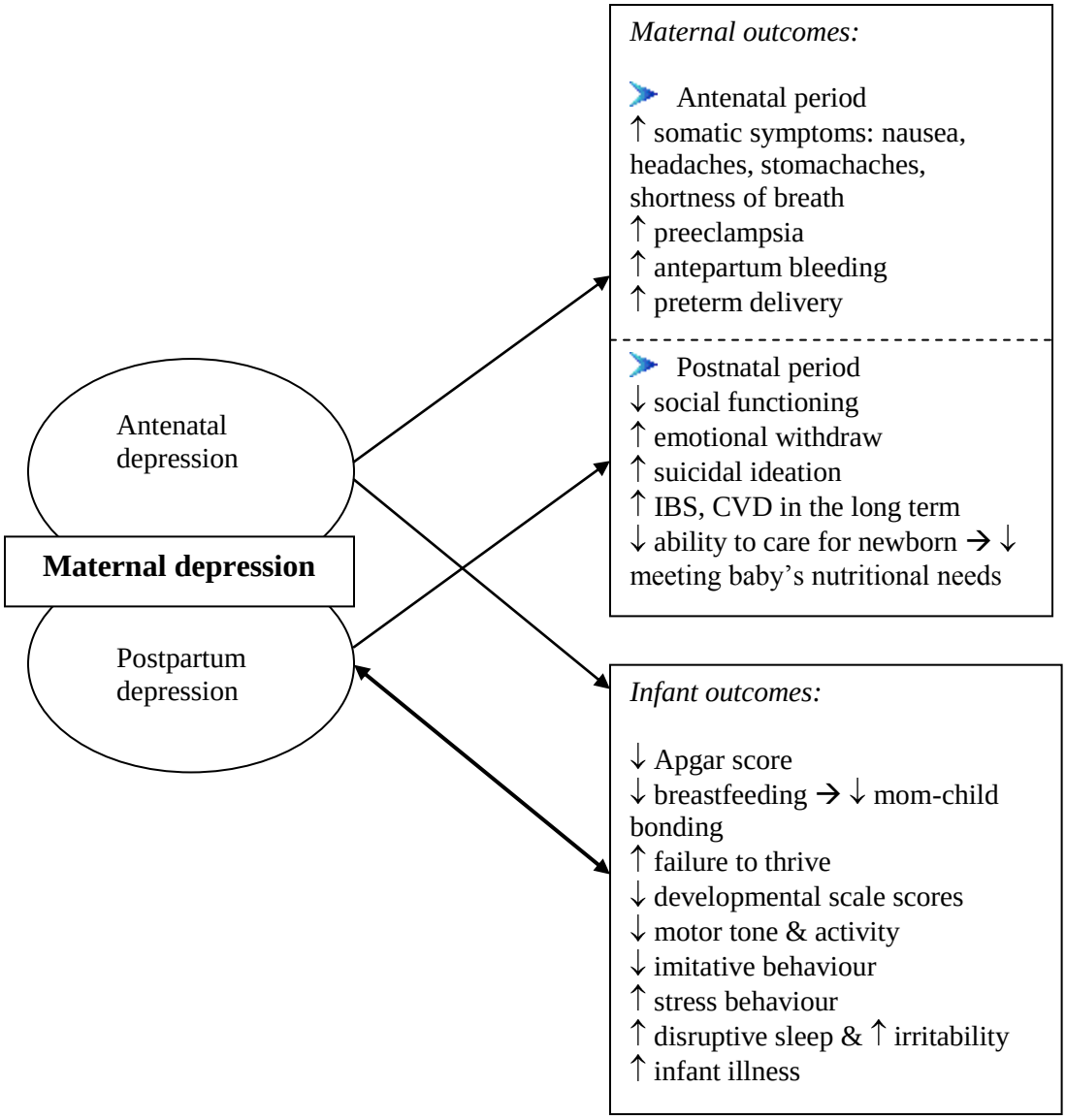
Study	Sample size	Measurement tool	Outcome
2006 (244)	women at 36 wks, 60 lactating women	records (for 3 d)	of lactating women did not meet folate requirements from dietary sources alone
Bodnar, 2007 (240)	200 white & 200 black pregnant women	Serum vitamin D status measured at 4-21 week gestation & cord blood of neonates	Vitamin D deficient* & insufficient** in 29.2% and 54.1% of black women and 45.6% and 46.8% black neonates, respectively. Five percent and 42.1% of white women and 9.7% and 56.4% of white neonates were vitamin D deficient & insufficient, respectively.
Marchioni, 2008 (172)	51 pregnant women, 100 age matched non-pregnant controls in Italy	urinary iodine concentrations (UIC) in morning spot urine samples	UIC lower than adequate in 92% of pregnant women compared with 4% of controls ($p < 0.001$).
Pinto, 2008 (245)	240 pregnant women in	Food questionnaire	low dietary intakes of vitamin E, folate and Magnesium both in the

Study	Sample size	Measurement tool	Outcome
	Portugal		preconceptional period and during pregnancy, and low intake of Iron during pregnancy

* *Deficient* is defined as hydroxyvitamin D [25(OH)D] $\leq$ 37.5 nmol/L

** *Insufficient* is defined in this study as 25(OH)D < 80 nmol/L; this cut-off correlates with a number of nutritional biomarkers that are impaired by inadequate vitamin D status (240)

Figure 3: Effects of maternal depression on maternal & infant outcomes (173)



CHAPTER 5: PRENATAL MICRONUTRIENT SUPPLEMENTATION AND POSTPARTUM DEPRESSIVE SYMPTOMS IN A PREGNANCY COHORT

***Leung MY Brenda**, Kaplan J Bonnie, Field J Catherine, Tough Suzanne, Eliasziw Misha, Gomez Fajer Mariel, McCargar J Linda, Gagnon Lisa, and the APrON Study Team. Prenatal micronutrient supplementation and postpartum depressive symptoms in a pregnancy cohort. BMC Pregnancy and Childbirth. 2013, 13:2. DOI: 10.1186/1471-2393-13-2.*

Abstract

Background: Postpartum depression is a serious problem for women and their offspring. Micronutrient supplements are recommended for pregnant women because of their documented protective effects for the offspring, but their potential beneficial effects on maternal mental health are unknown. This study investigated the association between prenatal micronutrient supplementation and the risk for symptoms of postpartum depression in a longitudinal pregnancy cohort from the Alberta Pregnancy Outcomes and Nutrition (APrON) study.

Methods: Participants came from a cohort of the first 600 APrON women. Supplemental nutrient intake and symptoms of depression (measured with the Edinburgh Postnatal Depression Scale (EPDS)) were collected at each trimester and 12 weeks postpartum.

Results: Of the 475 participants who completed the EPDS at least twice in pregnancy and at 12 weeks postpartum, 416 (88%) scored <10 and 59 (12%) scored $\geq$10, where an EPDS $\geq$10 is considered to be "at least probable minor depression". Mean nutrient intakes from supplements were higher in women with lower EPDS scores, particularly selenium ($p = 0.0015$) and omega-3s ($p = 0.01$). Bivariate analyses showed that several demographic and social/lifestyle variables were associated with EPDS $\geq$10: not having been born in Canada ($p = 0.01$), greater number of chronic conditions ($p = 0.05$), greater number of stressful life events during this pregnancy ($p = 0.02$), and lower prenatal and postnatal support ($p = 0.0043$ and $p = 0.0001$, respectively). Adjusting for covariates and nutrients known to be associated with postpartum depression, logistic regression showed that having a prenatal EPDS $\geq$ 10 increased the odds of postpartum depressive symptoms (second and third trimester OR = 3.29, 95% CI = 1.55 - 7.01, $p = 0.004$ and OR = 4.26, 95 CI = 2.05 - 8.85, $p < 0.0001$, respectively), while prenatal supplemental

selenium (per 10 µg, OR = 0.76, 95% CI = 0.74 - 0.78, p = 0.0019) and postnatal social support (OR = 0.87, 95% CI = 0.78 - 0.97, p = 0.0015) were protective.

Conclusions: Multiple factors, including supplementary selenium intake, are associated with the risk of postpartum depressive symptoms. Future research on dietary supplementation in pregnancy with special attention to selenium intake is warranted.

Keywords: postpartum depression, dietary supplements, selenium, omega-3

Introduction

Postpartum depression is a serious mental health problem that has been estimated to affect 10 - 15% of women after the birth of a child (22), and can occur anywhere from shortly after birth to one year postpartum. A review by Gavin and colleagues stated the prevalence may be as high as 19% within the first three months of giving birth (11). The difference in rates may be due to the diagnostic criteria, timing of screening, and screening instruments used (22). Regardless of the precise prevalence rate, it is clear that postpartum depression poses a significant public health issue because of its impact not only on the lives of the women themselves, but also on their children's growth and development (cognitive, social, behavioural) (184, 185).

A number of social and biological factors are known to be associated with an increased risk for developing postpartum depression. Social risk factors include a history of depression/anxiety, lack of a marital partner, marital difficulties, poverty, and lack of social support (194), as well as family violence, life stress, and substance abuse (252). Biological risk factors associated with postpartum depression include hormonal influences (200) and nutrient deficiencies from malnutrition or poor diet quality (4, 209, 210). Research has shown a number of nutrient deficiencies associated with depression in the non-pregnant or postpartum population, including folate and vitamin B12 (63, 212), calcium (212, 213), iron (63, 212, 214), selenium (63, 215), zinc (63, 217) and omega-3 polyunsaturated fatty acids (63-65, 93, 95, 219-221). Postpartum depression is not qualitatively different from depression that is not linked to pregnancy, but only recently has research on the role of nutrients in postpartum depression begun to emerge (168). A summary has been published elsewhere describing some of the known

biochemical effects of these nutrients on brain and neurological function, pointing toward the neurophysiological mechanisms by which insufficient nutrients could influence mood (168).

It is well-documented that proper nutrition during pregnancy is vital to the health of a woman and her fetus (70, 71). Today, it is common for health professionals to recommend that pregnant women take micronutrient supplements, the benefits of which have been established for the offspring. For instance, a meta-analysis by Goh and colleagues reported that offspring of women who took prenatal multivitamins were less likely to have leukemia (odds ratio (OR)=0.61, 95% confidence interval (CI)=0.50-0.74), pediatric brain tumors (OR=0.73, 95% CI=0.60-0.88) and neuroblastoma (OR=0.53, 95% CI=0.42-0.68), compared to children whose mothers did not take supplements (253). Another meta-analysis found prenatal supplements to be associated with decreased risk for a number of congenital anomalies (e.g. cardiovascular defects, limb defects, urinary tract anomalies, and cleft palate or oral cleft anomalies), in addition to neural tube defects (254). A meta-analysis of research in developing countries showed that multiple micronutrient supplementation was more effective than iron and folic acid supplementation at reducing the risk of low birth weight (RR=0.86, 95% CI=0.79-0.93) and of small size for gestational age (RR=0.85; 95% CI=0.78-0.93), but had no effect on perinatal mortality (255).

These results demonstrate important protective effects for the offspring from maternal use of prenatal multiple micronutrient supplementation. The effect of prenatal supplementation on maternal mental health, however, has not been established. The *purpose* of this study was to investigate the association between prenatal micronutrient supplementation and the risk for postpartum depressive symptoms in pregnant women from the Alberta Pregnancy Outcomes and Nutrition (APrON) study. We evaluated the nutrients ingested through supplements to determine

whether any individual supplementary nutrients were associated with postpartum depressive symptoms as measured by the Edinburgh Postnatal Depression Scale (EPDS).

Methods and Materials

Study design and participants

Participants for this study are the first 600 pregnant women from the APrON study, which is a longitudinal prospective study in Alberta, Canada. Participants were at least 16 years old with gestational age ≤27 weeks. Women must be in the first (T1) or second (T2) trimester to be in this study; we did not include any woman who was 28 weeks or beyond. Non-English speakers, known drug and alcohol abusers, and those planning to move out of the region within 6 months were excluded. Data were collected using questionnaires and interviews at each trimester and 12 weeks postpartum. Windows for data collection were defined *a priori* as week 10 ±2 for first trimester, week 18 ± 2 for second trimester, and week 32 ± 2 for third trimester. Every attempt was made to meet these specific timepoints as closely as possible (see Table 4). Details of recruitment and data collection are available elsewhere (256); also refer to the APrON website at www.ApronStudy.ca.

Participant background and covariates

Data on sociodemographic background and variables such as stressful life events and perceived social support known to be associated with increased risk for postpartum depression were collected using a questionnaire developed specifically for the APrON study (see Table 9). Perceived social support was measured with questions from the National Population Health Survey (NPHS) – Social Support section (Statistics Canada, 1994/95 & 1996/97 cycles) (118) with response options modified for the APrON study. The NPHS-Social Support section is comprised of four statements about having "someone to confide in", "someone one can count on

in a crisis", "someone one can count on for advice", and "someone who makes one feel loved and cared for", with Yes/No response options. Guided by expert advice from team members, the APrON study kept the wording of the statements, but modified the response options to five possible answers: none of the time, a little of the time, about half of the time, most of the time, all of the time. Each response option was then assigned a numerical value, "none of the time" was assigned a value of 0 and ranged up to a value of 4 for "all of the time". By summing the scores of each statement, an overall score of 16 indicated the highest possible level of perceived social support, while an overall score of 0 was the lowest possible level of perceived social support.

Prenatal nutrient intake from supplements

The Supplement Intake Questionnaire was developed for APrON to assess natural health product (NHP) intake. The structure of the Supplement Intake Questionnaire was adapted from questionnaires used in other studies of supplement intake, such as the National Cancer Institute and the Canadian Community Health Survey (257, 258). The Supplement Intake Questionnaire was pilot tested with a panel of nutrition experts as well as 50 pregnant women to determine whether it captured NHP data efficiently and accurately. Wording, formatting, and other modifications were made based on the feedback from the pilot study.

The final version of the Supplement Intake Questionnaire consisted of three sections: 1) section 1 listed options for multivitamins/minerals, 2) section 2 listed single nutrient supplements, and 3) section 3 consisted of herbal products, probiotics, homeopathic remedies, and other products such as amino acids, essential fatty acids and traditional medicines. Commonly used formulations were presented in a checklist. For products used by participants but not in the checklist, space was provided to record the name and manufacturer of the NHP as

well as the Natural Product Number (NPN) or Drug Identification Number (DIN). Where possible, the products were verified using their NPNs and DINs against those within Health Canada's Natural Health Products Database, to confirm their formulations; then they were entered into the APrON database. A NPN or a DIN is assigned to all NHPs that have met standards for quality, safety and efficacy set by Health Canada (259). Products that did not have a NPN or DIN were verified by obtaining ingredients from product labels or by finding the information from the manufacturer/supplier websites.

At each visit during pregnancy and postpartum, women were asked to describe in detail the quantity (i.e., frequency of intake and dosage) and type (e.g., prenatal multivitamins) of NHP consumed, using open-ended questions. To determine dose, women were asked "how much do you take?" and for frequency, women were asked "how often do you take it?" Typical responses were daily or times per week (e.g., 5 x/week). There was no menu of responses from which to choose. For example, if a woman said she consumed 700 mg/week of a nutrient, it was calculated as 100mg/day for that specific trimester. The daily value was calculated for the specific trimester, and only for that trimester, and not extrapolated for the entire pregnancy.

A trained Research Assistant conducted the interview with each woman to elicit the information. All information provided by the participants was reviewed with them to ensure the items were correctly recorded. Women were also asked to bring in bottles and other containers of the supplements they were taking. Brand names, individual nutrients and their amounts, as well as dosage (i.e. number of pills/capsules/tablets taken per day) were recorded. Prior to each visit, an email was sent to the women, reminding them to bring in the NHP container(s) and other materials to the next clinic visit. When a woman did not bring in the container(s) of the NHPs, the research assistant followed the visit with a phone call to obtain the information.

For the purpose of this study, only supplements of vitamins, minerals, and fatty acids were analyzed. Supplement data was collected a minimum of two times, and maximum of three times during pregnancy. To obtain an average intake of each nutrient in the supplements, reported intake was averaged over the number of times data were collected. For example, if information on vitamin D supplement intake was collected at each trimester (i.e., three times), then the total amount consumed was divided by three; if information was collected at only two times, then the total was divided by two. The nutrients chosen for analysis in this study (listed in Table 10) were informed by the literature that has previously demonstrated a relationship with mood (4). The Recommended Dietary Allowance (RDA) values from the Institute of Medicine (260) were used as the reference values for calculating the percentage of women above or below RDA for consuming the individual micronutrients from supplements. The comparison to RDA in this study did not include nutrients from dietary intake, but from supplement intake only.

Depression symptom measure – EPDS

The EPDS is a 10-item scale that measures mood, and requires about five minutes to complete. It has been widely validated, widely utilized, has a moderate to good reliability and test-retest reliability and has a good to moderate correlation with other depression measures (111). It has a maximum score of 30; a score of 10 or more indicates possible depression of varying severity. The standardized and validated EPDS has been used extensively worldwide, and has been evaluated for its psychometric rigor (20, 110, 112). It has been found to have high sensitivity and specificity (79% and 97% for first trimester at cut-off of 11, 70% and 96% for second trimester with cut-off of 10, and 76% and 94% for third trimester with cut-off of 10 (261)) in identifying those at risk of, or potentially suffering from prenatal depression.

The EPDS has been used for both clinical and research purposes, and cut-off scores have been obtained through empirical assessment (116, 261). These cut-offs provide rates of probable depression for referral or treatment, and can also be used to track the course of depression from antenatal to postpartum periods. As with other screening measures, sensitivity and positive predictive value may be variable when used in selected populations versus community populations (111, 112). For the purpose of this study, we used a cut-off score of 10 for probable depression (116). Using the sample size of 397 we had 90% power to detect a relative risk of 1.5 or more for EPDS >10 in women consuming lower vs. adequate RDA of a nutrient from supplements.

Assessing predictors of depressive symptoms on the EPDS

From the first model, only *born in Canada* was statistically significant (OR = 2.27, 95% CI = 1.20 - 4.31, p = 0.012). In the second model, only postpartum social support was statistically significant (OR = 0.83, 95% CI = 0.72 - 0.95, p = 0.007). A third model with the nutrient variables resulted in only selenium being statistically significant (OR = 0.97, 95% CI = 0.94 - 0.99, p = 0.030). Although omega-3 was not significant in the logistic regression (OR = 1.00, 95% CI = 0.99 - 1.00, p = 0.07), given the strong association of omega-3 with depressive symptoms, both selenium and omega-3 were included in the next model with the significant variables from the first two models.

The fourth model was constructed to include *born in Canada, prenatal and postnatal social support, prenatal EPDS* (at 2nd and 3rd trimesters) and *prenatal omega-3* and *selenium* supplement. Prenatal EPDS scores were included because the literature has reported that prenatal depressive symptoms were predictive of postpartum depressive symptoms. Born in Canada, prenatal social support, and omega-3 were removed from the fourth model as they exceeded the

pre-set p value of 0.05. Potential confounding of the remaining model by sociodemographic and lifestyle variables was assessed. No meaningful differences were found between the crude and adjusted ORs. Therefore, the final model consisted of predictors that were statistically significant (see Table 11). Hosmer and Lemeshow's goodness-of-fit test resulted in $chi^2(7) = 8.08$, and $p = 0.33$, indicating that our model fits the data well. The mean VIF = 1.13, with VIF for individual variable ranged from 1.03 to 1.17 (and tolerance from .84 to .97), indicating that multicollinearity was not likely to be a problem in the model.

Handling Missing values

To assess whether the 125 "missing" postpartum EDPS participants differed from those who responded, various statistical tests were used, including 1) exclusion of cases with missing postpartum EPDS, 2) re-categorization of missing as "not depressed", 3) re-categorization of missing as "depressed", 4) last observation carried forward using third trimester data, 5) creation of a category called "missing" to see what predicted "missing", and 6) comparison of participant characteristics with those without postpartum EPDS data. No difference was found with regard to the sociodemographic characeristics listed in Table 9 for those with completed EPDS compared to those with missing EPDS at the postpartum period.

Statistical methods

All statistical analyses were performed using STATA11 software. Values were expressed as mean and standard deviation, range, or proportions (n and %). Comparisons between groups (those having EPDS scores <10 and those with EPDS scores ≥10) were assessed for statistical significance by a Chi-square test for categorical variables and a t-test for continuous variables. Bonferroni correction was used for multiple test comparisons. Several logistic regression models were constructed to identify predictors of postpartum depression. Using multivariate logistic

regression, the first model consisted of all the demographic variables; model two included social/lifestyle variables plus the significant variable from model one. The third model assessed nutrients from supplement intake (listed in Table 10), and the fourth model incorporated significant (and close to significant) variables from models one, two, and three. For the final model, we tested the fit of the model with Hosmer and Lemeshow's goodness-of-fit test, and we computed the variance inflation factor (VIF) to test for collinearity. Odds ratios (OR) and the 95% CI for predictors of the EPDS depressive symptoms were calculated. A two-tailed p-value of 0.05 was considered statistically significant.

Ethics approval

The APrON study was approved by the Conjoint Health Research Ethics Board at the University of Calgary, and the Health Research Ethics Board at the University of Alberta. Each participant signed a consent form at the first clinic visit.

Results

Of the 600 pregnant women in the study cohort, 475 completed the EPDS questionnaire at least twice during pregnancy and at 12 weeks postpartum. Of the 125 with no EPDS data postpartum, nine miscarried, 36 withdrew from the study, 38 were non-respondent (i.e., could not be contacted), and 36 did not complete the EPDS. No differences in characteristics were found between those with postpartum EDPS data and those without.

Of the 475 available participants, 416 (88%) scored <10 on the EPDS and 59 (12%) scored ≥10. For demographic characteristics, women not *born in Canada* were more likely to have an EPDS ≥10 ($p = 0.01$); no other potential covariates were statistically significant (see Table 9). For the social/lifestyle characteristics, the *number of chronic conditions, stressful life events during this pregnancy,* and *pre/post-natal support* were all statistically significant (see

Table 9); i.e. EPDS was associated with these characteristics. Women with higher number of stressful life events 12 months before pregnancy and before the age of 17 were more likely to have EPDS ≥10, with p-values close to significance (p = 0.08 and 0.06 respectively). Previous self reported history of depression (1.7% suspected; 9.5% diagnosed) and postpartum depression (4.0% suspected; 3.5% diagnosed) were assessed as possible predictors for postpartum EPDS ≥10 in a separate analysis, and were found not to be statistically significant. Not presented here were regressions for prenatal depression and the key nutrients found to be significantly associated with postpartum depression: the results (not shown) were that selenium was not a predictor of prenatal depression at second or third trimesters. Although not statistically significant, a trend was shown for women being single/divorced, had lower income, or had higher number of children to be associated with EPDS ≥10.

Nutrient data

Almost all women (99%) took some type of micronutrient supplement during the prenatal period. For this cohort, the nutrients most commonly consumed were vitamins B6, B9 (folate), B12, and E, with more than 90% above RDA. The supplement taken the least was omega-3, where 68.5% of women with EPDS <10 and 78.0% of women with EPDS ≥10 did not take any omega-3. The mean intake of *selenium* and *omega-3* differed significantly (p = 0.0015 and 0.01, respectively) between women with EPDS <10 and those with EPDS ≥10. As well, the mean intakes for all the other nutrients listed in Table 10 were more likely to be higher in women with EPDS <10 than those with EPDS ≥10, although not statistically significant. In fact, the upper ranges for intake were higher in almost all nutrients (except for folic acid) in women with EPDS <10 than women with EDPS ≥10. Finally, all the other nutrients listed (except vitamin D and zinc) showed an overall nonsignificant trend for those consuming below RDA levels to be more

likely to have EPDS ≥10 (see Table 10). Supplement intake for many of the nutrients was not normally distributed. SIQ data was available for n=136 in the first trimester, n=575 in the second trimester and n=516 in the third trimester.

Discussion

In this study, postpartum depressive symptoms were found to be associated with multiple factors. Women with an EPDS score ≥10 were more likely to be born outside of Canada; to report having more chronic health conditions, more life stress and less social support during the current pregnancy; and to have consumed fewer micronutrients from supplements, most notably selenium and omega-3. Adjusting for covariates and nutrients known to be associated with postpartum depression, the logistic regression showed that prenatal EPDS ≥10 (at second and third trimesters) increased the odds of postpartum depressive symptoms, while prenatal selenium intake from supplements and postnatal social support were protective (decreased the odds) of postpartum depressive symptoms. It may also be meaningful that of the nutrients evaluated, all intakes were likely to be higher in women with EPDS <10 than those with EPDS ≥10. The results of this research support previous studies on the nutritional and social factors associated with depression (103, 194, 262) and are consistent with the finding that multiple factors are involved the development of postpartum depression.

The role of selenium in relation to mood is not as well studied as some other nutrients (e.g. omega-3s, folate, and zinc) that have been associated with depression. However selenium research is gaining more attention over the last couple of decades (263, 264). A recent nested case-control study by Pasco and colleagues found low intake of selenium (<8.9 μg/MJ/day) was associated with almost a three-fold increase in the likelihood of major depressive disorder (OR 2.95, 95%CI 1.00-8.72) after adjusting for age and socio-economic status (105). A randomized

trial by Mokhber and colleagues reported significantly lower mean EPDS scores in the group of primigravid pregnant Iranian women, aged 16 to 35, taking selenium supplements compared to a comparison group after controlling for sociodemographic and health history variables (103). Serum selenium concentration was also found to be significantly higher in the supplement group (n = 83) compared to controls (n = 83) in that research (103). However, in the non-pregnant population, the results have been mixed. A study by Rayman and others did not find any effect of selenium supplement on mood in a group of elderly participants aged 60 – 74 (106). On the other hand, Hawkes and Hornbostel in a study of eleven healthy men found lower selenium status associated with worse mood scores (265).

The biochemistry and mechanism of action of selenium in the brain has been well documented elsewhere (215, 266). Selenium (as selenoproteins) is known to be neuroprotective, and selenium concentrations may modulate the status of various neurotransmitters. Benton reviewed the literature on selenium and its association with aging, mood, and psychological functioning and reported that the brain had preferential retention of selenium (215). Selenium is bound to proteins as selenoproteins in the circulation, and is essential in the make-up of specific enzymes such as selenoprotein glutathione peroxidase, with anti-oxidant function. The antioxidant property of selenium is believed to mediate the oxidative damage implicated in a number of psychiatric disorders, including depression (215). Selenium is also required for the synthesis and metabolism of thyroid hormones; for example, selenophosphate synthetase is involved in normal thyroid function (215). Thus, selenium is believed to modulate thyroid function, and thyroid status has been associated with the likelihood of depression (215, 266). While the research into the role of selenium on neurological and psychological health may be

limited, the findings from this study provide support for further research on the function of selenium in relation to mood and mental health in pregnancy.

We had expected omega-3 fatty acid supplementation to be inversely related to EPDS scores (i.e. increased omega-3 intake associated with decreased risk of depressive symptoms) given the ample evidence on this topic (77). The absence of support for this inverse relationship in our data may be due to the small number of individuals who took omega-3 supplements (29.4%) compared to the proportion of the sample taking selenium (83.6%). Grigoriadis and colleagues reported similar results in their study, in which over 90% of pregnant women took a multivitamin but only 11% took omega-3 supplements (267). Furthermore, the average intake for this population was far below the therapeutic dose suggested in the literature ($\geq$ 2 g/d) (268).

This study reinforced the importance of social support for pregnant women in the prenatal and postnatal periods, the benefits of which have been well-documented for the reduction of the risk and symptoms of mood disorders and depression (262, 269). For example a study by Joseph and colleagues on the relationships of chronic stress and social support with symptom reduction and remission in depressed patients treated with antidepressant medication found increases in social support from baseline to last visit were associated with more symptom reduction and higher likelihood of remission than individuals taking medication alone (270). While there is little research into the mechanism by which environmental factors such as social support affect brain biochemistry in humans, animal studies indicate that improving external stimuli through an enriched environment may reverse the effects of stress-related events, at the behavioral, endocrine, and biochemical levels (271). In other words, factors such as social support may mitigate the effect of stress events on depressive symptoms.

The findings from this study also revealed the need for evaluation for prenatal depressive symptoms. In recent years, the estimated prevalence of postpartum depression has become better known in healthcare, resulting in the creation of programs in many jurisdictions to evaluate new mothers for the risk of this condition. However, prenatal mental health still receives little attention as part of prenatal care (272, 273). Since antenatal depression may be more common than postnatal depression, and women with antenatal depression are more likely to be depressed postnatally (274), prenatal depression screening should be as widely practiced as postpartum screening.

One limitation of this study was the effect of multicollinearity as a number of variables were closely correlated (e.g. various B vitamins with each other, and with vitamin C and E). Multicollinearity is a common problem in nutrition studies, particularly those that examine the intake of nutrients from supplements as supplements include multiple nutrients and many are in similar amounts and combinations due the nutrient recommendations by health agencies. Another limitation is that the present study did not have access to serum levels of selenium or any of the other nutrients discussed in this paper, thus there is no information as to whether supplementation affected biological levels. A third limitation was that dietary (food) intake was not included in the analysis. Although dietary intake was estimated in these women at two or three trimesters during pregnancy (data which will be available at some future date), selenium intake is difficult to estimate due to variations in the food supply and the inaccuracy of nutrient databases. The high supplement intake may mitigate dietary intake in this case, and would not be likely to change our findings. We did not have lab values at present to assess anemia in our sample. Although anemia is known to be a risk factor for depression, we also know that women are routinely supplemented with iron when anemia is diagnosed during pregnancy. In this study,

for women below RDA, 82.9% scored <10 EPDS, 17.1% scored ≥10 EPDS; for women above RDA, 88.5% <10 EPDS, and 11.5% were ≥10, p = 0.84 (see Table 10), indicating that there was no difference of scoring ≥10 EPDS between being above or below the RDA for iron. While we recognize this is not the ideal measure for iron associated form of anemia, it served as a proxy for iron status. There is also a potential effect on our findings of differences in macronutrient intake. Another limitation was possible reporting error associated with recall of supplement intake (e.g. dosage and frequency). Recording of brand names and labels of supplements (e.g. types and amounts of nutrients) by research assistants, as well as the use of repeated measures, helped to minimized potential reporting bias. However, we acknowledge a major limitation with the Supplement Intake Questionnaire is self-reporting; thus we did not know whether the women actually consumed their supplements as they said they did. Furthermore, if a woman missed her trimester visit then this was treated as missing data and no estimation was made. Thus, there was some degree of projection as the women were asked for information for the entire trimester at the trimester midpoint and not the end, making this a limitation to our estimates. A limitation in the analysis was that we did not assess for interaction among the nutrients due to the absence of any *a priori* rationale from the literature. In addition, as data on prenatal nutrition status was not available, we were unable to test whether the effect of prenatal micronutrient supplementation on postpartum depression varied by prenatal nutritional status. For future research, we would like to assess whether specific nutrients interact to impact postpartum depression, and also whether prenatal nutritional status modified the association of supplementation and postpartum depression.

One of the strengths of this study was the ability to collect data prospectively over the prenatal and postnatal periods. Thus, the temporal relationship of supplement intake and

postpartum depressive symptoms could be established. Another strength was that supplement intake was collected repeatedly over time to obtain an average usual intake of the nutrients in the prenatal period, rather than using a single measure as in most studies. In addition, the nutrients were recorded in detail, getting container labels, and verifying ingredients. A third strength was the ability to control for a large number of factors associated with postpartum depressive symptoms in our analyses because of the variables (demographic, social, lifestyle) collected prospectively. The evaluation of multiple variables associated with postpartum depression demonstrates that biological and social risk factors are involved, and thus multiple solutions, services (e.g. social support), and therapies (e.g. nutrient supplementation and/or drug therapy) may be needed in a therapeutic plan.

The findings from this study support the evidence that there are multiple factors that influence postpartum depression. There is growing evidence that intake of multiple nutrients are beneficial not only to the developing fetus, but also to the mother's well-being. In addition to the common recommendation of folic acid to prevent neural tube defects, clinicians may want to consider selenium for its benefits on mood. Implications for future research include examining objective measures of pre- and postnatal micronutrient status for: 1) relations between pre/postnatal blood nutrient levels (or changes in pre/postnatal blood levels) and postpartum depression, 2) moderating effect of prenatal blood nutrient levels on the relations between prenatal micronutrient supplementation and postpartum depression, and 3) interactions amongst nutrients and their effects on postpartum depression.

Conclusion

This study found multiple factors, including selenium intake from supplements, history of depression, and social support, are associated with the risk of postpartum depressive symptoms.

The evidence suggests that there is likely no single casual factor to depression, and nutrients play an important role in the development of depressive symptoms. Thus, a future research focus on dietary supplementation with special attention to the intake of selenium in the pregnant population is warranted.

Acknowledgments

We are extremely grateful to all the families who took part in this study and the whole APrON team (www.ApronStudy.ca), including our research assistants, graduate and undergraduate students, volunteers, clerical staff and managers. This cohort was established by an interdisciplinary team grant from Alberta Innovates – Health Solutions (formerly Alberta Heritage Foundation for Medical Research). BL is the recipient of the Scobey Hartley Doctoral Award from the Alberta Centre for Child, Family and Community Research.

The individual members of the APrON research team are Bonnie J. Kaplan, Catherine J. Field, Deborah Dewey, Rhonda C. Bell, Francois P. Bernier, Marja Cantell, Linda M. Casey, Misha Eliasziw, Anna Farmer, Lisa Gagnon, Gerald F. Giesbrecht, Laki Goonewardene, David W. Johnston, Libbe Kooistra, Nicole Letourneau, Donna P. Manca, Jonathan W. Martin, Linda J. McCargar, Maeve O'Beirne, Victor J. Pop, Nalini Singhal

Competing interest

All authors declare no conflict of interest, financial or otherwise.

Authors' Contributions

BMYL carried out the data analyses and drafted the manuscript. BJK reviewed and revised the manuscript. LJM and MFG provided methodological information on the supplement intake questionnaire. CJF, ST, and LG consulted on measures of nutrition, sociodemographics, and mental health. ME provided input on statistical analysis. All authors read and approved the final manuscript.

Table 9: Associations between socio-demographic characteristics of women and their postpartum EPDS

Characteristic	EPDS<10*	EPDS≥10*	p-value**	Odds Ratio*** (95% CI)
Demographics				
Age (mean (95% CI))	31.2 (30.8 - 31.6)	31.6 (30.3 - 32.8)	0.51	1.0 (0.9 - 1.1)
BMI (mean (95% CI))	24.1 (23.6 - 24.5)	24.2 (22.9 - 25.4)	0.90	1.0 (0.9 - 1.0)
Marital status (n (%))				
Married/common-law	406 (88.0)	55 (11.9)	0.17	1.0
Single/divorced	9 (75.0)	3 (25.0)		2.4 (0.6 - 9.3)
Education (n (%))				
High school	36 (87.8)	5 (12.2)	0.96	1.0
Trade/undergrad	286 (87.7)	40 (12.2)		0.6 (0.1 - 7.0)
Graduate	93 (87.7)	13 (12.2)		0.6 (0.1 - 5.9)
Household Income (n (%))				
< $20K	5 (83.3)	1 (16.7)	0.13	1.0
$20-$39K	12 (80.0)	3 (20.0)		1.2 (0.1 - 15.1)
$40-$69K	47 (78.3)	13 (21.7)		1.4 (0.1 - 12.9)
$70-$99K	107 (87.7)	15 (12.3)		0.7 (0.1 - 6.4)
≥$100K	236 (90.1)	26 (9.9)		0.5 (0.1 - 4.9)
Born in Canada (n (%))				
Yes	349 (89.9)	39 (10.0)	0.01	1.0
No	63 (79.7)	16 (20.2)		2.3 (1.2 - 4.3)
Ethnicity (n (%))				
Caucasian	363 (88.3)	48 (11.6)	0.30	1.0
Non-causasian	51 (83.6)	10 (16.4)		1.5 (0.7 - 3.1)
# of children (n (%))				
0	57 (87.7)	8 (13.3)	0.28	1.0
1-2	172 (88.2)	23 (11.8)		1.0 (0.5 - 1.9)
3-4	4 (66.7)	2 (34.3)		7.3 (1.0 - 53.7)
Social/lifestyle				

Characteristic	EPDS<10*	EPDS≥10*	p-value**	Odds Ratio*** (95% CI)
# of chronic conditions (n (%))				
0	206 (85.1)	36 (14.9)	0.05	1.0
1	91 (91.9)	8 (8.1)		0.5 (0.2 - 1.1)
2	15 (78.9)	4 (21.0)		1.5 (0.5 - 4.8)
3	5 (62.5)	3 (37.5)		3.4 (0.8 – 15.0)
# of life event stresses (n (%))				
During this pregnancy				
0	207 (89.6)	24 (10.4)	0.02	1.0
1-2	184 (88.0)	25 (11.9)		1.2 (0.6 - 2.1)
>3	24 (72.7)	9 (27.2)		3.2 (1.3 - 7.7)
12 mos before pregnancy				
0	196 (89.9)	22 (10.1)	0.08	1.0
1-2	149 (88.2)	20 (11.8)		1.2 (0.6 - 2.3)
>3	13 (72.2)	5 (27.8)		3.4 (1.1 - 10.5)
Before age 17				
0	126 (89.3)	15 (10.6)	0.06	1.0
1-2	224 (89.2)	27 (10.7)		1.2 (0.6 - 2.6)
>3	11 (68.7)	5 (31.2)		3.8 (1.1 - 12.5)
Social support (mean (95% CI))				
Prenatal	14.8 (14.7 - 15.0)	13.8 (13.1 - 14.5)	0.0043	0.8 (0.7 - 0.9)
Postpartum	14.7 (14.5 - 14.9)	13.0 (12.2 - 13.8)	0.0001	0.8 (0.7 - 0.9)
EPDS score by time points (mean (95% CI)				
Time A (1st trimester)	4.8 (4.1 - 5.6)	8.0 (6.5 - 9.5)	0.0006	1.31 (1.11-1.55)
Time B (2nd trimester)	5.0 (4.7 - 5.4)	8.4 (7.2 - 9.5)	<0.0001	1.21 (1.13-1.30)
Time C (3rd trimester)	4.5 (4.2 - 4.8)	9.0 (7.9 - 10.1)	<0.0001	1.33 (1.23-1.44)
Time E (12 weeks postpartum)	3.6 (3.4 - 3.8)	12.1 (11.4 - 12.9)	<0.0001	n/a

* EPDS≥10 = “at least probable minor depression”. The frequency calculations differed by variable because of missing values in some cases (e.g. participant did not answer a question).

**Assessment of associations with Chi-square test for categorical variables or t-test for continuous variable.

***OR from univariate logistic regression.

Table 10: Comparison of prenatal nutrient intake from supplementation in pregnant women and their postpartum EPDS

Nutrient	EPDS<10 (n=416)	EPDS≥10 (n=59)	p-value*
Vitamin B1 (mg)			
Mean (SD)	5.5 (14.7)	3.5 (8.1)	0.12
Range	0 – 103	0 – 62.5	
Below RDA (n (%))	55 (84.6)	10 (15.4)	
Above RDA (n (%))	361 (88.1)	49 (11.9)	
Vitamin B3 (mg)			
Mean (SD)	22 (23)	19 (10)	0.10
Range	0 – 335	0 – 66	
Below RDA (n (%))	55 (82.1)	12 (17.9)	
Above RDA (n (%))	361 (88.5)	47 (11.5)	
Vitamin B6 (mg)			
Mean (SD)	9.1 (15.9)	7.8 (10.7)	0.43
Range	0 – 110	0 – 62.5	
Below RDA (n (%))	23 (79.3)	6 (20.7)	
Above RDA (n (%))	363 (88.1)	49 (11.9)	
Folate B9 (µg)			
Mean (SD)	1259 (924)	1297 (962)	0.78
Range	0 – 6000	0 – 5000	
Below RDA (n (%))	30 (81.1)	7 (18.9)	
Above RDA (n (%))	386 (88.1)	52 (11.9)	
Vitamin B12 (µg)			
Mean (SD)	31 (116)	18 (65)	0.23
Range	0 – 1210	0 – 502	
Below RDA (n (%))	19 (79.2)	5 (20.8)	
Above RDA (n (%))	397 (88.0)	54 (12.0)	
Vitamin C (mg)			
Mean (SD)	147 (188)	124 (104)	0.17
Range	0 – 2083	0 – 585	
Below RDA (n (%))	40 (81.6)	9 (18.4)	
Above RDA (n (%))	376 (88.3)	50 (11.7)	
Vitamin D (IU)			
Mean (SD)	618 (539)	567 (512)	0.48
Range	0 – 4200	0 – 2750	
Below RDA (n (%))	292 (87.7)	41 (12.3)	
Above RDA (n (%))	124 (87.3)	18 (12.7)	
Vitamin E (mg)			
Mean (SD)	28 (44)	25 (26)	0.43
Range	0 – 625	0 – 210	
Below RDA (n (%))	36 (81.8)	8 (18.2)	
Above RDA (n (%))	380 (88.2)	51 (11.8)	

Nutrient	EPDS<10 (n=416)	EPDS≥10 (n=59)	p-value*
Iodine (µg)			
Mean (SD)	173 (68)	158 (76)	0.15
Range	0 – 636	0 – 330	
Below RDA (n (%))	227 (85.7)	38 (14.3)	
Above RDA (n (%))	189 (90.0)	21 (10.0)	
Iron (mg)			
Mean (SD)	33 (20)	32 (20)	0.84
Range	0 – 127	0 – 87	
Below RDA (n (%))	68 (82.9)	14 (17.1)	
Above RDA (n (%))	348 (88.5)	45 (11.5)	
Magnesium (mg)			
Mean (SD)	67 (65)	59 (39)	0.19
Range	0 – 1025	0 – 208	
Below RDA (n (%))	415 (87.5)	59 (12.5)	
Above RDA (n (%))	1 (100.0)	0	
Selenium (µg)			
Mean (SD)	25 (17)	19 (13)	0.0015
Range	0 – 125	0 – 45	
Below RDA (n (%))	401 (87.2)	59 (12.8)	
Above RDA (n (%))	15 (100.0)	0	
Zinc (mg)			
Mean (SD)	13 (8)	12 (8)	0.38
Range	0 – 48	0 – 32	
Below RDA (n (%))	233 (87.9)	32 (12.1)	
Above RDA (n (%))	183 (87.1)	27 (12.9)	
Omega-3 (mg)			
Mean (SD)	180 (440)	90 (208)	0.01
Range	0 – 4050	0 – 1000	
Below RDA (n (%))	RDA not determined	RDA not determined	
Above RDA (n (%))			

Note: RDA (Recommended Dietary Allowance) for pregnant women is "the average daily nutrient intake level sufficient to meet the nutrient requirement of nearly all (97– 98%) healthy individuals in a particular life stage and gender group" as defined by the Institute of Medicine (55).

SD = Standard deviation, * p values derived from t-tests

Table 11: Full and final models of predictors for EPDS depressive symptoms (EPDS≥10)

Predictor	Odds Ratio	95% CI	p-value
Model 1			
Age	.98	.88-1.09	.69
Parity	.98	.56-1.71	.94
Marital status	.49	.19-1.24	.14
Education	.98	.63-1.51	.92
Income	5.32	.85-33.35	.07
Born outside Canada	2.41	.81-7.19	.11
Ethnic background	.73	.19-2.79	.65
BMI	1.00	.88-1.15	.90
Model 2			
Life stressors during this pregnancy	1.21	.87-1.69	.26
Life stressors 12 mons before this pregnancy	1.08	.77-1.50	.65
Life stressors prior to age 17	1.22	.85-1.75	.29
Social support	.85	.74-.97	.015
Model 3			
Vitamin B1	.98	.94-1.02	.33
Vitamin B3	.99	.97-1.01	.41
Vitamin B6	1.01	.98-1.05	.41
Vitamin B9 (folic acid)	1.00	.99-1.00	.96
Vitamin B12	1.00	.99-1.00	.76
Vitamin D	1.00	.99-1.00	.92
Iodine	.99	.99-1.00	.11
Iron	1.00	.98-1.01	.91
Magnesium	.99	.99-1.00	.37
Selenium	.97	.95-0.99	.03
Zinc	.98	.95-1.02	.41
Omega 3	1.00	.99-1.00	.20
Final model			
Prenatal EPDS (2nd trimester)	3.29	1.55 – 7.01	0.004
Prenatal EPDS (3rd trimester)	4.26	2.05 – 8.85	0.000
Postpartum Social Support	0.87	0.78 - 0.97	0.015
Prenatal Selenium supplement intake (per 10 µg)	0.76	0.74 - 0.78	0.019

CHAPTER 6: MICRONUTRIENTS AND CHILD NEUROCOGNITIVE DEVELOPMENT

Introduction

The previous chapters have presented some of the growing evidence on the importance of micronutrients to the mental health of pregnant women. Ensuring adequate or sufficient micronutrient intake during pregnancy, thus sufficient nourishment during crucial stages of development, is believed to be beneficial also for the foetus. Micronutrient supplements have been recommended to pregnant women for many years, and women appear to be heeding the advice. The Prenatal Health Project, London, Ontario, found 79.9% of pregnant women reported taking one or more dietary supplements containing either iron, zinc, or folic acid (275). In the Maternal Experience Survey, 89.7% of women reported taking supplements during the first three months of pregnancy (23). In particular, women who were older, with higher level of education, primiparous, and above the low income cut-off (<$40,000) were more likely to take supplements (23). An analysis of the APrON cohort found about 99% of pregnant women took some form of supplement, from single nutrient to multiple nutrients, consisting of over 400 different brand names (see Chapter 5). In fact many APrON women surpassed the RDA (and the UL in some cases) for a number of nutrients (e.g. such as folic acid, vitamins B6 and B12, and vitamin E) with more than 90% of women consuming above RDA for those nutrients (see Chapter 5).

While researchers are learning about the impact of supplementation on maternal mental health, the effect of supplementation on the developing foetus, in particular on brain (and consequently, cognitive and behavioural) development, is still relatively new. This chapter examines the micronutrients that have been studied in connection to neurodevelopment. The following is a summary of the theories about the possible mechanisms by which micronutrients

affect neural development and function, and the evidence that maternal micronutrient intake/status affects neurocognitive development in the offspring.

Nutrients are fundamental to many aspects of brain health and function, such as cell membrane integrity, metabolites and enzyme functions, and neurotransmitter actions (276). The brain grows rapidly (more so than the rest of the body) during the third trimester and the first two years of life. Thus, variations in nutrient availability (i.e. clinical and possibly subclinical deficiencies) could result in changes to brain structures that could lead to long-lasting consequences in cognition and behaviour (277). The brain is especially dependent on specific nutrients during critical periods of development (277). For example, it has been well documented that the effects of iron and iodine deficiency during foetal development include reduced cognitive capability for life (277). Folate, probably the most studied vitamin in terms of neural development, is crucial in brain development for its role in nucleotide synthesis, DNA integrity and transcription; thiamine (B1) plays an important role in brain energy metabolism and in myelin synthesis; pyridoxine (B6) as a coenzyme is essential for the production of the neurotransmitters serotonin and noradrenaline; cyanocobalamine (B12) is significant in the formation of erythrocytes, DNA synthesis, and fatty acid metabolism in the production of myelin; choline is a precursor for phospholipids and the neurotransmitter acetycholine, and choline is believed to be associated with the risk of neural tube defects due to its role in stem cell proliferation and apoptosis (277). There is growing evidence that omega 3 fatty acids increase membrane flexibility and protein-lipid interactions of nerve cells, thus enhancing neuronal activity and cognition (278). Omega 3 fatty acids may also be protective against inflammatory processes, oxidative stress, and cytokine activities. Another proposed mechanism for DHA is that it regulates brain-derived neurotrophic factor and nerve growth factor, which are required for

normal neurological development (279). Other nutrients of particular neurological importance are trace minerals such as copper and zinc, which are critical in neurotransmitter and DNA synthesis (278).

Early nutrition may have profound effects on the mental health of individuals later in life (278). Studies have linked inadequate intake of certain nutrients to the increased incidence of cognitive and behavioural disorders such as depression, learning disability, attention deficit, autistic spectrum disorder, to name just a few (278). The following sections provide greater detail about the impact of micronutrients on neurodevelopment.

Folic acid

As stated above, folate (or folic acid) has been well studied since its role in preventing neural tube defects was established (280). Folate is the naturally occurring form found in foods, while folic acid is the synthetic form. A comprehensive review of the positive effects, sides effects, and interactions of folic acid has been provided by the Natural Medicines Comprehensive Database, U.S. National Library of Medicine (281) (website: http://www.nlm.nih.gov/medlineplus/druginfo/natural/1017.html). In addition to decreasing the risk of neural tube defects, folic acid has been shown to be beneficial for a number of other conditions, see Figure 4 (281).

Figure 4: Beneficial effects, sides effects, and interactions of folic acid

Conditions for which folic acid likely to be effective

- Lowering homocysteine levels ("hyperhomocysteinemia") as high levels of homocysteine have been linked to heart disease and stroke, including people with kidney disease who are prone to have high levels of homocysteine (about 85%);
- Reducing harmful effects of a medicine called methotrexate, which is sometimes used to treat rheumatoid arthritis and psoriasis. Taking folic

acid seems to reduce nausea and vomiting, which are possible side effects of methotrexate treatment.

- Reducing the risk of getting colorectal cancer, i.e. lowers the risk of developing colon cancer, but no effect in people already diagnosed;
- Reducing the risk of breast cancer where the benefit is greater when women get extra vitamin B12 and vitamin B6 in their diet in addition to folic acid.
- Reducing the risk of pancreatic cancer.
- Reduce the risk of macular degeneration when taken with vitamin B6 and vitamin B12.

Side effects of folic acid supplementation

- Acute symptoms related to high doses of folic acid could include abdominal cramps, diarrhea, rash, sleep disorders, irritability, confusion, nausea, stomach upset, behavior changes, skin reactions, seizures, gas, and even excitability.
- Excess folic acid (i.e., 800-1200 mcg) for a prolonged period of time has been associated with increased risk of heart attack in people who have heart problems as well as increased risk of cancer such as lung or prostate cancer.

Interactions between folic acid and medications

- 5-Fluorouracil where large amounts of folic acid may increase some side effects of 5-fluorouracil, especially stomach problems.
- Capecitabine (Xeloda) where large amounts of folic acid might increase the side effects of capecitabine, especially stomach problems like diarrhea and vomiting.
- Fosphenytoin (Cerebyx) where folic acid can increase the breakdown of fosphenytoin (Cerebyx), thus might decrease the effectiveness of fosphenytoin (Cerebyx) for preventing seizures.
- Methotrexate (MTX, Rheumatrex) is a folic acid antagonist, thus folic acid would decrease the effectiveness of methotrexate (MTX, Rheumatrex) in relieving the symptoms of psoriasis and rheumatoid arthritis.
- Phenobarbital (Luminal) where folic acid can decrease how well phenobarbital (Luminal) works for preventing seizures.
- Phenytoin (Dilantin) where folic acid might increase the breakdown phenytoin (Dilantin), and thus might decrease the effectiveness of

phenytoin (Dilantin) and increase the possibility of seizures.
- Primidone (Mysoline) where folic acid might decrease how well primidone works for preventing seizures.
- Pyrimethamine (Daraprim) where folic acid might decrease the effectiveness of pyrimethamine (Daraprim) for treating parasite infections.

Other Possible Interactions

- There is no known interaction with foods
- There is limited evidence that green tea might impair the function of folic acid in the body, possibly leading to a condition similar to folic acid deficiency.

Now research is looking at the association between folate and child neurodevelopment, particularly in terms of cognition and behaviour. The Generation R study found that children whose mothers were folate deficient were 1.5 times (95% CI: 1.03 to 2.38) more likely to have emotional (but not behavioural) problems (282). The study also found a higher risk for emotional problems in children whose mothers started folic acid supplementation later in the pregnancy (e.g. third trimester) or not at all than in children whose mothers took folic acid prior to pregnancy (OR = 1.45, 95% CI: 1.14 to 1.84) (282). Another study from the Generation R cohort examined self-reported folic acid supplementation in the first trimester in relation to child behavioural development at 18 months, as measured by the Child Behaviour Checklist (283). The study found that children whose mothers did not take folic acid supplements were at a higher risk of having a high total problem score (OR = 1.44; 95% CI: 1.12 to 1.86). In general, children of mothers who did not take folic acid supplements (median serum folate =11.1 (95% CI: 4.7 to 29.6) nmol/l) were more likely to suffer from both internalising (OR = 1.65; 95% CI: 1.24 to

2.19) and externalising problems (OR = 1.45; 95% CI: 1.17 to 1.80), compared to children whose mothers took folic acid (median serum folate of 23.5 (95% CI: 4.3 to 45.3) nmol/l) (283).

A Spanish birth cohort with 420 children examined the link between folic acid supplementation in the first trimester and psychological outcome of the children at age four (284). In this study, women were asked about supplement intake, but timing of when intake started, duration or dosage of intake was not provided. For the analysis, children were classified into 1) "no folic acid or other supplements", 2) "use of folic acid containing supplements", and 3) "use of vitamins without folic acid" (284). Children of mothers supplemented with folic acid were more likely to have higher scores in verbal, motor, and verbal-executive function, better social competence, and fewer inattention symptoms (284). A Norwegian birth cohort reported that maternal use of folic acid supplements in early pregnancy (first trimester) was associated with a reduced risk of severe language delay in children at age 3 years (285). In this study, the amount of folic acid intake was not provided, and the children were also divided in four comparison groups: (1) no use of dietary supplements; (2) other supplements, but no folic acid; (3) folic acid only; and (4) folic acid in combination with other supplements (285).

Other studies have examined the role of folate in behaviour disorders such autistic spectrum disorder (ASD). The CHARGE (CHildhood Autism Risks from Genetics and Environment) Study in the U.S. found periconceptional folic acid (>600 μg/d) may reduce ASD risk in those with inefficient folate metabolism (286). Schmidt and colleagues reported the mean folic acid intake in the first month of pregnancy was significantly greater for mothers of non-ASD children than for mothers of children with ASD (779.0 ± 36.1 and 655.0 ± 28.7 μg, respectively; $p < 0.01$), and the risk estimates decreased with increased folic acid (p-trend = 0.001) (286). Furthermore, the role of genetics was also assessed and the association between

folic acid and reduced ASD risk was strongest for mothers and children with MTHFR 677 C>T variant genotypes (286). The role of genetic variant on folate metabolism was supported by a study on maternal genotypes of MTHFR (677C>T and 1298A>C), folate and B12 intake, and child neurodevelopment (287). del Río Garcia and colleagues measured maternal dietary intake of vitamin B12 and folate during the first trimester of pregnancy, and then evaluated mental and psychomotor development in 253 children at age one using the Bayley test (287). They found dietary B12 intake (dosage and frequency not provided) was negatively associated with mental development (beta = -1.6; 95% CI: -2.8 to -0.3), while dietary intake of folate (< 400 mg/day) reduced the mental development index only among children of mothers who were carriers of the TT genotype of the MTHFR gene (beta = -1.8; 95% CI: -3.6 to -0.04) (287). Thus, the impact of perinatal folate intake on child neurodevelopment was particularly important in populations that were genetically susceptible (287).

An intriguing explanation of the possible mechanism by which folate influences neurodevelopment was presented by Villamor and colleagues, who proposed that methyl-donor nutrients (i.e. folate, vitamin B12, choline, betaine and methionine) are substrates for methylation reactions involved in neurodevelopmental processes (288). The study, from the birth cohort Project Viva in Massachusetts, examined the associations of maternal intake of folate, vitamin B12, choline, betaine and methionine, using a 166 item food frequency questionnaire in the first and second trimesters of pregnancy, with tests of cognitive performance in the offspring at age three using the Peabody Picture Vocabulary Test III (PPVT-III) and visual-motor skills with the Wide Range Assessment of Visual Motor Abilities test (288). For each 600 µg/day increment in total folate intake (maximum amount not provided by authors) during the first trimester, PPVT-III score at age 3 years was 1.6 points (95% CI: 0.1 to 3.1; p = 0.04) higher.

There was a weak inverse association between vitamin B12 intake during the second trimester and PPVT-III scores (-0.4 points per 2.6 μg/day; 95% CI: -0.8 to -0.1; p = 0.01) (288). No associations were found between choline, betaine or methionine and cognitive outcomes (288).

Iodine

Another nutrient associated with brain development and function is iodine. Iodine deficiency is a common problem globally, and its effect on foetal development, as well as the short and long term consequences after birth, has been well studied (289). Iodine is crucial to proper thyroid function, and consequently plays an important role in mental and motor development. During foetal development, maternal thyroid hormones are the only source for foetal thyroid hormone concentrations until birth (290). While overt iodine deficiency is known to cause cretinism, characterised by profound mental retardation, subclinical iodine insufficiency may also impair neurodevelopment (290). Skeaff assessed neurodevelopment in children of moderately iodine deficient mothers and found that supplementing mothers earlier rather than later in pregnancy was beneficial (290). Moderate maternal iodine insufficiency may be associated with learning disabilities in the children (291).

There have been conflicting results regarding maternal iodine intake/status and child neurodevelopment, as seen in the next two studies. In a Spanish birth cohort study, Murcia and colleagues assessed the association between maternal iodine intake from diet and supplements during pregnancy with infant neurodevelopment at age one using the Bayley Scales of Infant Development (292). Iodine intake and supplement were assessed using a semiquantitative food frequency questionnaire administered at 10–13 weeks and at 28–32 weeks (292). The study reported that maternal intake of >150 μg/day of an iodine supplement was associated with lower psychomotor achievement: i.e. a 5.2-point decrease in Psychomotor Development Index (PDI) of

the Bayley and a 1.8-fold increase in the odds of a PDI <85 (292). No difference was found in the Mental Development Index of the Bayley (292). When stratified by sex, the effect was significant in girls (OR=4.0 95% CI: 1.4, 11.4, p = 0.017), but not boys (292). Maternal urinary iodine concentration, iodized salt consumption, or dietary intake of foods with high iodine content were not associated with infant neurodevelopment (292). In this study, it appears that excessive levels of iodine can have negative consequences for the foetus. Intake was obtained from self report using a 100-item food frequency questionnaire adapted from the Willet questionnaire that was previously validated and developed for an adult Spanish population (292). Conversely, in a pseudo-experimental study by Velasco and colleagues, a convenience sample of pregnant women were given 300 μg of iodine in the form of potassium iodide (n = 133) in the first trimester of their pregnancy (293) and continued during lactation; a control group (n = 61) receiving no supplement was drawn from a pool of women in their last month of pregnancy attending the same hospital. When the children were tested with the Bayley Scales of Infant Development at age two, children of the supplemented group had significantly higher scores on the PDI and the Behaviour Rating Scale (293) of the Bayley compared to the non-supplemented group. The inconsistent findings between the Murcia and Velasco studies may be a result of differences type of supplementation, and timing of supplementation. As Murcia and colleagues acknowledged, a major limitation to their study was that the iodine was part of multi-nutrient supplement, and thus they could not rule out the effects of other nutrients on the outcome (292).

Iron

Another micronutrient that is important to reproductive health is iron. Iron plays an important catalytic role in many cytochromes (compounds consisting of heme bonded to a protein) and enzymes (biological molecules that catalyze specific biochemical reactions)

involved in fatty acid desaturation (e.g. removal of a hydrogen molecule with the insertion of a double bond), especially during pregnancy, when cell proliferation and differentiation are very active (294, 295). Iron-deficiency in newborns has been shown to result in altered recognition memory, and behavioural problems; furthermore the memory deficits can last well into adulthood in spite of iron repletion (294, 296). Sub-optimal iron status during pregnancy has also been linked to an increased risk of mental problems such as schizophrenia (295, 297). An observational study by Rioux and colleagues found no relationship between maternal iron or DHA status and infant cognitive development (298). Of the pregnant women in the Rioux study, 9.5% were anaemic, 34.9% had low iron stores, and 3.2% suffered from iron deficiency anemia (298). Another observational study by Tamura and colleagues measured umbilical cord serum ferritin concentrations and tested the children at age 5 (299). They reported that children in the lowest quartile for ferritin status scored lower on every test and had significantly worse language ability, fine-motor skills, and tractability (i.e. ability to obey rules and follow general orders of decorum) (299). They were also 4.8-fold more likely to score poorly in fine-motor skills and 2.7-fold more likely to have poor tractability than children in the median quartiles (299). However, another intervention study in Bangladesh supplemented infants with iron, zinc, iron+zinc, or a micronutrient mix, and found weekly iron and zinc supplementation had beneficial effects on motor development and orientation-engagement, but iron alone did not (300).

Omega 3 Fatty Acids

The n-3 polyunsaturated fatty acids (PUFA), in particular, docosahexaenoic acid (DHA), has gained much attention for its role in neurodevelopment, but the intervention studies have so far yielded inconsistent results. In a study of a Spanish birth cohort, maternal fish intakes of >2-3 times/week were associated with significantly higher scores on several subscales of the

McCarthy Scales of Children's Abilities compared with intakes ≤ 1 time/week (301). A clinical trial of healthy pregnant women from Spain, Germany and Hungary were randomly assigned to take fish oil (500 mg/d DHA + 150 mg/d EPA), 400 μg/d 5-methyltetrahydrofolate, both, or placebo from week 20 of gestation until delivery, and neurological functioning of the children was tested at age 5.5 years (302). The study found an increase in the neurological score with every unit increment in cord blood DHA level at delivery (95% CI: 1.09 to 2.26) (302). In contrast, another clinical trial in the Netherlands assessed the effects of DHA (220 mg/day, n=41), DHA+AA (arachidonic acid) (220 mg/day, n=39) or placebo (n=34) during pregnancy and lactation on neurodevelopment at 18 months, and found no differences across groups (303). van Goor and colleagues concluded that relatively short-term maternal DHA or DHA+AA supplementation did not influence neurodevelopment at toddler age (303).

Arachidonic acid has been included with DHA in supplements for a number of reasons. Arachidonic acid is synthesized in the body from linoleic acid (an omega-6 essential fatty acid), and is essential to mediating numerous physiological and biochemical processes along with DHA and EPA. Animal studies that used DHA alone, without AA, have resulted in negative growth and cognitive development (304). In fact, the importance of DHA and AA for visual and cognitive development in full term infants is reflected in the DHA and AA levels of human (breast) milk, which makes up 0.32% and 0.47% of total fatty acids, respectively (304). It is well known that DHA and AA are needed for rapid synthesis of cell membranes, in particular, of neural cells (305).

A review by van de Rest and colleagues indicated beneficial effects of PUFA on brain development, brain functioning and depression, but the results have not yet been replicated in randomized controlled trials (79). A systematic review by Lo and colleagues reported most

studies did not show a sustained benefit for maternal PUFA intake (supplement) on infant cognition or visual development (306). Lo and colleagues also reported that among the studies, there were variations in dosage (some used insufficient amounts to impart an effect on foetal brain), time of supplementation (some missed periods of crucial brain development), and outcome measurements (assessed different parts of brain development leading to inconsistent results) (306). These differences may account for the inconsistent results from clinical trials.

Vitamin D

A nutrient that is gaining increasing attention for its effects on general health is vitamin D. In particular, the relationship between vitamin D and neurodevelopment is starting to emerge. A review by Eyles and colleagues described vitamin D as a neuroactive steroid due to a number of unique characteristics (see Table 12) (307).

Table 12: Characteristics of vitamin D as a neuroactive steroid (307)

1. The vitamin D receptor is widely distributed in the brain, and is expressed at critical periods during development; as well, vitamin D metabolites are known to cross the blood brain barrier;
2. Vitamin D signalling is involved in neuronal cell proliferation and differentiation in the brain;
3. Vitamin D regulates calcium in the brain by blocking the hyperpolarizing and/or toxic effects of calcium influx in embryonic neurons;
4. Vitamin D may increase potent anti-oxidants such s glutathione, and block neuronal uptake of reactive species such as hydrogen peroxide;
5. Vitamin D may influence neuronal development via its effects on

neurotrophic factor production; in rat studies, vitamin D has been shown to increase the synthesis of nerve growth factor which is essential for the growth and survival of cells in the brain;

6. Vitamin D as an anti-inflammatory factor may be protective to the developing brain; some evidence has linked inflammation (which may mediate the adverse effects of infection) in the developing brain to increased risk of schizophrenia and autism.

Thus, the impact of vitamin D in the developing brain is a result of multiple possible mechanisms related to the integrity, connectivity, and function of neuronal and non-neuronal cells (308). However, the evidence on these mechanisms is based on *in vitro* studies; thus, the clinical implications are still untested. Given that vitamin D insufficiency (25(OH)D < 80 nmol/L) and deficiency (hydroxyvitamin D [25(OH)D] $\leq$ 37.5 nmol/L) (240) may be relatively common, particularly in pregnant women, the relationship of vitamin D and (foetal) neurodevelopment requires comprehensive investigation.

Studies on maternal vitamin D levels and child neurocogitive developmental outcome are limited. A longitudinal study by Whitehorse and colleagues assessed maternal serum levels of vitamin D during pregnancy and offspring's neurocognitive development using the Child Behaviour Checklist at 2, 5, 8, 10, 14, and 17 years of age, and the Peabody Picture Vocabulary Test-Revised (for receptive language) at ages 5 and 10 years (309). Whitehorse reported no significant association between serum vitamin D quartiles and offspring behavioural/emotional problems at any age, but there were significant linear trends between quartiles of maternal vitamin D levels and language impairment at ages 5 and 10 (309). In fact, the study found that a

child whose mother had vitamin D insufficiency (≤46 nmol/L) during pregnancy was almost twice as likely to have language difficulties compared with children of women whose vitamin D levels were >70 nmol/L (309).

Vitamin D has been linked to ASD, and the risk factors are similar to those for schizophrenia discussed below (307). An epidemiological study by Grant and Sole, looking at seasonal variation of birth rates and latitude for children born before 1985, found an association between vitamin D levels and risk for infantile autistic disorder (310). Other studies have reported children with ASD were more likely to have low vitamin D levels compared to children without ASD (311-313).

A review by Eyles and colleagues reported on studies that linked seasonality of birth (i.e. winter), higher altitude, and migration of dark-skinned individuals to northern climates to the increased risk of schizophrenia (307). The commonality in these factors appeared to be hypovitaminosis D (307). A number of studies by McGrath and colleagues showed a trend (although not statistically significant) of low vitamin D levels and increased risk for schizophrenia (314-316).

Vitamin A

Finally, a study examined the correlation of vitamins A, E and C in maternal and cord blood at delivery and child neurodevelopment at age two (317). Zhang and colleagues reported vitamin A was positively associated with motor development quotients (DQ) and average overall DQ ($p<0.01$), as well as positively related with language and social DQ ($p<0.05$) (317). No association was found with vitamin E or C (317). The mechanism behind how vitamin A may have an effect on child neurodevelopment has yet to be determined.

Research questions

As presented in this chapter, many nutrients are known to affect cognitive and behavioural processes. Several nutrients are known to be crucial to neuronal function and synaptic plasticity, and ultimately, brain health and mental function (276). While the focus has been the micronutrients, we recognize that nutritional balance and nutrient interactions, from overall dietary intakes, energy balance, macronutrients, fatty acids, vitamins, and trace elements, are fundamental to health (278). By understanding the critical role of nutrition on brain health, we have the potential to optimise brain function, prevent dysfunction, and treat disease (278). Given the evidence provided here, the question we would like to address in the next chapter is: *What is the strength of the evidence that perinatal supplementation improves neurocognitive development in children?*

CHAPTER 7: DOES PRENATAL MICRONUTRIENT SUPPLEMENTATION IMPROVE CHILDREN'S MENTAL DEVELOPMENT? A SYSTEMATIC REVIEW

***Leung BMY**, Wiens KP, Kaplan BJ. Does prenatal micronutrient supplementation improve children's mental development? A systematic review**.** BMC Pregnancy and Childbirth 2011, 11:12 (3 February, 2011).*

Abstract

Background: Although maternal nutrient status influences all aspects of fetal development including the brain, the impact of micronutrient supplementation on the baby's mental function is a topic of debate. This systematic review assesses the effect of single and multiple micronutrient supplementation during pregnancy on offspring mental development. **Methods:** Eleven electronic literature databases were searched using key terms of various combinations and filter string terms. Reference lists of articles selected for review were scanned for citations fitting the same inclusion criteria. Each stage of the literature retrieval and review process was conducted independently by two reviewers. The CONSORT checklist was used to assess study quality. **Results:** A total of 1316 articles were retrieved from the electronic database search, of which 18 met the inclusion criteria and were evaluated. The selected studies were randomized controlled trials published from 1983 to 2010, with high variance in sample size, intervention type, and outcome measures. The median CONSORT score was 15 (range 12 – 19). Due to inconsistent interventions and outcome measures among the studies, no conclusive evidence was found that enhancing the intrauterine environment through micronutrient supplementation was associated with better child development. Multi-micronutrients and n-3 fatty acids appeared to have some positive effect on mental development. **Conclusions:** The study of children's mental outcomes as a function of prenatal supplementation is still relatively new, but the results of this systematic review suggest that further work with multiple micronutrients and/or n-3 fatty acids should be conducted.

Background

Almost universally, governments and health professionals suggest that pregnant women take prenatal formulas containing various micronutrients to ensure a healthy pregnancy and healthy baby. In addition to these general guidelines, the more specific use of B-vitamins (especially folic acid) is now commonly recommended, as they reduce the risk of neural tube defects if consumed during the periconceptional period. The impact of such perinatal supplementation is well described for variables involving physical health, including birth outcomes, fetal growth, and infant physical development (318, 319). For instance, a review by Shah *et al.* (320)concluded that pregnant women supplemented with multi-micronutrients had significantly lower risk for low birth weight (though not for prematurity) compared to women supplemented with only iron and folic acid.

Guidelines for prenatal supplementation are of considerable importance beyond the immediate obstetrical implications. The long-lasting health impact of the intrauterine environment on the developing foetus has become an area of investigation ever since Barker demonstrated the link for cardiovascular disease and type II diabetes (321). One well-recognized component of the intrauterine environment that is likely responsible for many of those health effects is nutrition. It is now understood that maternal nutrient status can influence fetal development in all phases, including brain development, which would affect behaviour and cognitive function (322). The support for an association between gestational nutrition and brain development has been particularly strong for iron, n-3 fatty acids, and folate (209, 210, 323).

In summary, there is no debate about the contribution of micronutrients to health, nor the importance of pregnant women eating well to maximize the outcomes for their babies. Even the concept of micronutrient supplementation for pregnant women appears to be a universally

accepted practice, and is widely accepted as 'insurance' to prevent adverse perinatal outcomes, especially in those at risk for inadequate nutrient status due to other health factors(324). However, the two topics that are still open to much investigation and debate are the optimal content of micronutrient supplementation, and whether there is a long-term impact on the baby's mental function.

The existing literature was systematically reviewed to assess the impact of prenatal supplementation on offspring mental development, including cognitive development, psychomotor abilities, intelligence, and behavior/temperament. This systematic review focused specifically on the effect of single and multiple micronutrient supplementation during pregnancy on offspring mental development.

Methods

Search Strategy

The search strategy selected randomized trials and cohort studies in humans, with English-only text, with no limitations set for date of publication. B.L. consulted a Research Librarian at the Centre for Health and Policy Studies, Community Health Sciences at the University of Calgary to develop the search strategy, the inclusion/exclusion criteria, abstract screening tool, keyword list, validated search filters, and databases.

Eleven electronic literature databases (period of coverage noted below) were searched by B.L. between Dec. 22 and 30, 2009: Medline/PubMed (1950 to November Week 3 2009), HealthStar (1966 to Nov. 2009), EMBASE (1980 to 2009 Week 52), PsychInfo (1967 to Dec. week 4, 2009), CAB Nutrition Abstracts (1973 to 2009 week 51), Cochrane Library (1991 to Nov. 2009), AMED (1985 to December 2009), ERIC (1965 to Nov. 2009), CINAHL (to week of Dec. 22, 2009), Scopus and Web of Science (to week of Dec. 22, 2009).

The search syntax included four key parts: 1) terms defining the population of interest (pregnancy, pregnant women, prenatal, perinatal, antenatal); 2) terms for micronutrients (supplement, micronutrient, dietary supplement, vitamin, mineral, folic acid/folate, iron, iodine, B complex, B_{12}, selenium, zinc, vitamin A, vitamin D); 3) terms for developmental outcomes (infant development, child development, mental development, brain development, neurodevelopment, cognitive development, psychomotor, IQ, behavior); and 4) terms for study design (randomized controlled trial (RCT), pseudo-experimental, clinical trial, longitudinal cohort). Validated filters were used in the search strategy to ensure that all possible design terms for RCTs and cohort studies were used. The Cochrane search filters were used for RCTs (325) and the BMJ Knowledge filters were used for observational studies (326, 327).

In addition, reference lists of articles selected for review were scanned for citations fitting the same inclusion criteria. This process enabled the identification of additional literature that may otherwise have been missed in the database search. An updated search was conducted August 9, 2010 to look for articles published since the initial search. One study was found (328) and was included in the review.

Inclusion Criteria

Randomized controlled trials (RCTs) that investigated the effects of single or multi-micronutrient supplementation during pregnancy on child development (including mental, cognitive, psychomotor, intelligence and behavior) were included. Other study designs such as pseudo-experimental and cohort studies were excluded because only one of each of the aforementioned designs was found and the application of the CONSORT scoring (see section below on quality appraisal) was inappropriate for comparison to RCTs. Also, those reporting

maternal nutrient intake or status (but not supplementation) and the effects on pregnancy or birth outcomes, and physical (but not mental) development or growth were excluded.

Identified citations were assessed against the inclusion criteria independently by two reviewers (B.L. and B.K.), first using the titles, then using the abstracts, and finally using the full text. Any disagreement was resolved by discussion with reexamination of the document, and a consensus was reached. Articles on visual development were included after a review of the abstracts revealed that it was being used as a measure of neural development.

Data Extraction and Quality Appraisal

Details of the studies were extracted by two reviewers (B.L. and K.W.) and summarized in tables. Key data elements extracted included subject and intervention characteristics, and outcomes of interest. Data from each accepted study were reviewed and extracted independently by B.L. and K.W.; quality assessment was determined by using the revised CONSORT 25-item checklist (329). Disagreements between the reviewers were resolved by discussion, and the consensus score was used for all analyses. The intraclass correlation coefficient for inter-observer agreement was calculated. Four CONSORT items were excluded because they were not applicable (19 (Harms), and 23 – 25 (Other information)), leaving a maximum score of 21 for each study (one point for each CONSORT item satisfied). The items were not weighted because the CONSORT statement is not a validated instrument. The articles were rated "good" quality if the score was $\geq$ 17 (meeting >80% of the checklist items), "average" if the score was between 13 – 16 (meeting 60-79% of the checklist items) and "poor" if $\leq$ 12 (meeting < 59% of the checklist items).

Results

A total of 1316 articles were retrieved from the electronic database search, of which 18 met the inclusion criteria and were included in the final review (Figure 5). Three trials reported only on cognitive development (330-332), three reported visual development outcomes (333-335), one reported behavior and temperament alone (336), and the remaining studies examined outcomes for cognitive, psychomotor, behavior, visual and/or auditory development (Table 13) in various combinations. The outcome measure most widely used was the Bayley Scales of Infant Development (seven studies) (337-343).

We had intended to prepare tables and bubble plots comparing effect sizes of studies, grouped according to potential effect modifiers (characteristics of the printed educational materials, complexity of behavior and quality of comparisons) to explore heterogeneity. However, given that few studies reported this information, comparing effect size was not possible. A meta-analysis of the studies was also considered, but given the heterogeneity across the studies and the outcome measures in the studies reviewed, a meta-analysis was not feasible.

Study Characteristics

In general, the variability in this small set of papers was unexpectedly high in virtually every dimension evaluated. The 18 studies reviewed were published from 1983 to 2010, and included pregnant women from 14 different countries (ranging from Australia to Tanzania), varying across rural and urban settings in developing and industrialized nations (Table 13). Sample size varied from 29 to over 2000 participants. The offspring were assessed from two months to nine years age.

The interventions (Table 14) ranged from a single micronutrient (four studies) (336, 337, 344, 345) to multi-micronutrients of various combinations (six studies) (328, 339, 343), as well

as n-3 fatty acids in the forms of cod liver oil (two studies) (330, 331), blended fish oil (three studies) (335, 338, 346), docosahexaenoic acid (DHA) in capsules (one study) (334), and DHA-containing cereal bars (two studies) (332, 333). Of the six multi-micronutrient formulas, no two were alike (see Table 15).

Exposure dose and time period also varied considerably, although there was greater agreement about treatment onset: most began prior to 26 weeks gestation. Several studies failed to specify the rationale used for the selection of the ingredients of their formula or the dose (337).

Follow up period also varied greatly among studies, from 60 days to 66 weeks for visual acuity tests, and 3 months to 7.5 years for developmental tests. A number of studies had repeated follow up time points where assessment was conducted. Some studies showed a positive outcome at one assessment time, and null outcome at another time (333, 341, 342). Outcome did not appear to be associated with length of follow up period.

Quality of Reporting

The total scores on the CONSORT checklist ranged from 12 (331, 332) to 19 (341), with a mean score of 14.95 ± 1.80 (the maximum possible score was 21). The median score was 15. Four studies had quality scores rated "good", 12 were rated "average", and two were "poor". The agreement between the pair of reviewers who independently assessed the RCTs using the CONSORT checklist was excellent (347) (ICC = 0.775; 95% CI = 0.491–0.910; P <0.001).

The majority of studies did not meet the CONSORT statement standards (329), and none of the studies reported all 21 items assessed. For example, only seven studies (38.9%) reported how sample size was determined, one of which determined sample size *post hoc*. While all of the studies reviewed were reported as RCTs, only 33% (n=6) provided information on generation of

the random allocation sequence, 22% (n=4) referred to concealment of the allocation sequence, and 11% (n=2) described the implementation of the randomization. Furthermore, only Judge *et al.* (332) and Innis and Friesen (334) reported on all three items relating to randomization, whereas nearly 70% (n=12) of the RCTs reviewed did not report any information regarding randomization. Information on blinding after intervention assignment was somewhat better reported, yet 38.9% (n=7) of the studies still failed to mention who was blinded to group assignment.

Of the four studies that rated "good" for quality, one study on multivitamin-mineral supplementation reported a positive outcome (341), and the other three reported no effect (336, 339, 345). Two studies on fish oil/DHA that rated "poor" on quality (331, 332) did report positive outcomes. Thus, there was no consistency between study quality and outcome.

Seven studies with a positive outcome had quality scores between 12 to 19 (331-333, 340-342, 346). Four of these studies had fish oil/DHA as the intervention, and three were multivitamin-minerals. Eleven studies had null or negative outcomes, with quality scores ranging from 14 to 17. In these 11 studies, six used a single micronutrient, four used fish oil/DHA, and one used multivitamin-mineral food packets. Furthermore, 10 studies were conducted in Western "developed" countries, of which four had positive outcomes and six had null/negative outcomes; eight studies were from "developing" countries in Asia, Eastern Europe, South America, and Africa, of which four had positive outcomes and four had null/negative outcomes. Quality scores for studies in "developed" countries ranged from 12 to 17, while for "developing" countries, the range was 13 to 19. Outcome did not appear to be associated with location of the study.

Mental Development Outcomes

Three trials reported improvements in cognitive development, each with different single and multi-nutrient supplements (331, 332, 341), yet the improvements were transient with respect to age groups tested in two of the studies. There was no consistency among these reports regarding the specific aspect of cognitive development that improved, whether processing, problem solving or overall cognitive development.

Psychomotor outcomes were improved in three studies: two involving multivitamin-mineral supplements (340, 342), and one with fish oil capsules (346). Both the multivitamin-mineral supplement studies found improved psychomotor scores using the Bayley Scales of Infant Development, whereas the study on fish oil (346) found improvements in hand-eye coordination.

There were no trials reporting significant differences between treatment and placebo groups among those assessing behavior or temperament. Visual development was improved in one study (333), yet this improvement was only transient: DHA supplementation was associated with improved visual acuity tested at four but not at six months of age. All other studies investigating visual development had null results.

Negative effects on developmental outcomes were reported in a small number of studies. Hamadani *et al.* (337) found that children whose mothers were supplemented with zinc prenatally scored lower on cognitive and psychomotor indices. Similarly, two studies which examined the effects of iron on neurodevelopment (336, 345) found higher, but non-significant, rates of abnormal behavior and peer problems in iron-supplemented groups compared with placebo, though this was only in a small subsample from each study.

Discussion

Principal Findings

This systematic review revealed no conclusive evidence that an enhanced intrauterine environment through nutrient supplementation was associated with better mental development in the child. While cognitive, psychomotor and visual function showed improved outcomes with supplementation in several studies, the findings were often transient (not detectable when children were tested later in life) with poor corroboration among studies.

Though not conclusive, there was some evidence to support supplementation with n-3 fatty acids or multiple (but not single) micronutrients having some positive effect on mental development. Among the n-3 fatty acid intervention studies, four reported higher scores in one or more outcome measure in the intervention group compared to the placebo group (331-333, 346), while four studies found no difference between the groups (330, 334, 335, 338). There were significant methodological limitations in some of the studies with no results, which may be responsible for the null findings. For instance, there was poor compliance with the n-3 fatty acid capsules in the study by Tofail *et al.* (338), and there was significant contamination in the 'placebo' group used by Helland *et al.* (330, 331), in that approximately 50% of children, regardless of group assignment, consumed cod liver oil regularly during the preschool years. Also, the dose and formulation employed by two of the studies (334, 335) was relatively low at 200 mg of only DHA.

There is epidemiologic evidence that maternal dietary n-3 fatty acid intake influences mental development. For example, Gale *et al.* (348) used the Strength and Difficulties Questionnaire and Wechsler Abbreviated Scale of Intelligence to examine the association between fish intake in pregnant women and their children's IQ at age 9. Compared to mothers

who did not eat oily fish during gestation, there was a reduced risk of hyperactivity in children whose mothers had eaten oily fish in early pregnancy and higher verbal IQs in those whose mothers ate fish in late pregnancy

Among the vitamin and mineral studies reviewed here, better cognitive and/or psychomotor outcomes were reported for supplementation when the intervention involved multiple micronutrients (340-342). In contrast, the results for single micronutrient supplementation of pregnant women on child neurodevelopment were null for iron (336, 345), folic acid (343), vitamin A (343), and zinc (328, 344). The positive findings with respect to multi-nutrient supplementation are supported in the literature. Wehby and colleagues (349), using survey data, found that prenatal multivitamin-mineral use was associated with reduced risk of language and social development delays during childhood, whereas single micronutrients showed variable effects, several of which were negatively associated with developmental outcomes.

Other correlational and epidemiologic studies of maternal nutrient status and child development have reported mixed and inconsistent results. Bhate *et al.* (350) did a follow-up comparison of cognitive function in 9-year-old children, and found that children of women with high plasma B_{12} during pregnancy performed significantly better on the Color Trail Test (subtest A) and Digit Span Test (backward), but not on the Raven's Colored Progressive Matrices and Visual Recognition tests. However, another follow-up study compared cognitive development in 5-year-old children as a function of maternal folate status during pregnancy, finding no differences in the test scores of neurodevelopment between the low folate and normal folate groups (351).

Given that maternal nutrient status and intake appear to be associated with infant outcomes in terms of physical health, and perhaps some indicators of mental function, should broader supplementation guidelines be considered? A number of studies have found nutrient inadequacies in pregnant women living in western countries consuming a typical western diet (168). A study by Ray *et al.* (8) reported that after a decade of folic acid fortification, other B-vitamin deficiencies, such as B_{12}, continue to occur in up to five percent of pregnant women. These authors concluded that B_{12} deficiency may be an independent risk factor for neural tube defect. Thus, even in developed, nutritionally-abundant countries, nutrient inadequacy or deficiency may be more common than realized.

Another aspect of nutrient requirement not considered is genetic variance. A report by Cavalli *et al.* (352) discussed the "folic resistance" hypothesis among some women. That is, while prenatal folic acid supplementation prevented about 70% of neural tube defects in one dataset (353), there are cases of folate-resistant and folate-sensitive NTD subtypes, which are supported by animal models (352). In a case series, Cavalli and colleagues (352) supplemented women with high NTD recurrence risk with periconceptional inositol and folic acid to prevent reputed folate-resistant fetal NTDs. The addition of inositol to folic acid appeared to prevent the recurrence of NTDs in subsequent pregnancies and deliveries. Thus, this is further evidence that multi-micronutrient supplementation during pregnancy may confer greater benefit than single nutrient supplementation for infant outcomes.

Limitations of Current Evidence

The primary limitations were related to the methodologies and reporting of the 18 studies included in this review: unclear recruitment, randomization, blinding, follow up, unclear or unspecified supplement dosage and reason for dosage setting, and lack of monitoring or

reporting of compliance. Other limitations we noted were small sample size, variability of follow-up periods, and inadequate information regarding factors such as home environment, education, and level of stimulation. These social and educational variables are important in that the children were exposed to them between birth and the time of final assessment, hence influencing outcomes.

There is also some question regarding the selection of placebos used in the n-3 fatty acid intervention studies. While a true placebo is biologically inert, n-3 fatty acid intervention studies provide the control groups with metabolically-active compounds in order to maintain visual appearance, namely oils with varying concentrations of fatty acids. One could argue that these oils cannot serve as a control supplement given their distinct effect on maternal lipid and fatty acid profiles (354). This use of metabolically active 'placebos' leads to uncertainty as to whether differences reported between n-3 fatty acids and 'placebo' groups can be attributed solely to increased n-3 fatty acid intake.

Few studies provided information pertaining to diet quality, whether by assessing nutrient status with blood samples or dietary intake through recalls or questionnaires. Thus, the adequacy of prenatal nutrient status was not known, limiting conclusions about how baseline dietary intake and status may affect infant mental development in supplementation trials. It is possible that if a woman has widespread nutrient deficiencies, supplementing with a single nutrient would not have any noticeable impact on the offspring's mental development, perhaps accounting for why multi-micronutrient supplements appeared to be associated with better outcomes.

Given the variability in study populations, interventions used, outcomes measured, and the overall low reporting quality of the studies, our findings cannot answer our *a priori* question nor be generalized to a broader population. This inconsistency among the studies is reflected in

the low CONSORT scores. The use of the CONSORT checklist to evaluate studies may not always provide an adequate appreciation of rigor, because low scores may be at least partly explained by the historical focus of CONSORT – pharmaceutical and treatment-based RCTs; thus, the applicability of CONSORT to RCTs of a non-pharmaceutical nature is unclear. However, the use of a checklist like the CONSORT provided a means to assess consistency of the studies in this systematic review, and highlighted a number of limitations that made interpretation problematic.

Strengths and Weaknesses of the Systematic Review

The initial search criteria employed here included only English language articles, and RCTs, pseudo-experimental and cohort studies, which may have resulted in missing some pertinent studies. Since much of the literature on the topic of gestational nutrition emerges from the developing world, the loss of some of the non-English literature may limit the generalizability of the conclusions that were drawn. The fact that a meta-analysis was not feasible is also a weakness that could not be overcome given the present status of the published research on this topic. On the other hand, this systematic review appears to be the first attempt to evaluate objectively the literature that is beginning to develop on the topic of prenatal supplementation and children's mental outcomes. Although many of the studies were published prior to the development of current methodologic standards (e.g., the CONSORT guidelines) and hence cannot be faulted for the weaknesses they manifest, the tabular presentation of those weaknesses at this point in time may be useful for guiding future studies in this area.

Conclusions

This review attempted to assess the state of evidence for the relationship between prenatal supplementation and infant mental development. We recognize that infant mental

development is the result of complex multifactorial processes. Nutrients form the bases for proper neural development and could have long-lasting impact on mental development later in life. Given that pregnant women are often told by primary clinicians to incorporate folate (with or without B_{12}) and/or iron into their diet, it is important to know that the research seems to indicate that single nutrient supplementation is less adequate than supplementation with more complex formulas. This finding was derived from studies from both developed and developing countries. Although not conclusive at this stage, the evidence suggests there is value in further research examining the potential benefit of prenatal multi-micronutrient and n-3 fatty acid supplementation for child mental development. Future studies should consider the timing, duration, and required dosage of supplementation that meets the needs of the developing foetus to fully examine the impact of multi-micronutrients (including n-3 fatty acid) on child mental development.

Competing interests

No potential conflict of interest was reported by the authors.

Authors' contributions

All authors have made significant contributions to this study and meet criteria for authorship.

Acknowledgements

The authors thank the Alberta Heritage Foundation for Medical Research (AHFMR), Alberta Mental Health Board, and the Alberta Children's Hospital Research Institute (ACHRI) for their ongoing support. The authors are also grateful to Diane Lorenzetti, Community Health Sciences Librarian at the University of Calgary, for her assistance in developing the search strategy. The authors thank Dr. Anna Farmer for her suggestions at the early stages of the systematic review. FUNDING SOURCE: BL is the recipient of the Canadian Institutes of Health Research (CIHR) Training Program in Genetics, Child Development and Health studentship 2010.

Table 13: Summary of articles included in systematic review

Reference	Sample	Outcomes	Measures	Intervention/Duration	Results	Quality Score
Caulfield et al. (328)	184 children (Peru)	1,3 (At 4-5 yrs)	Wechsler Preschool & Primary Scale of Intelligence Goodenough & Harris Draw-a-Person Test Vineland Adaptive Behaviour Scales Preschool Behaviour Questionnaire	Zinc + folic acid + iron vs. folic acid + iron only ▪ 10-14 weeks gestation to birth	▪ Ø 1,3	15/21
Dunstan *et al.* (346)	72 children (Australia)	1,2,3 (At 34 mo)	Griffiths Mental Development Scale Peabody Picture Vocabulary Test Child Behavior Checklist	Fish oil vs. olive oil ▪ 20 weeks gestation to birth	▪ Ø 1,3 ▪ + 2	16/21
Hamadani *et al.* (337)	168 children (Bangladesh)	1,2,3 (At 39 mo)	Bayley Scales of Infant Development Wolke's Behaviour Rating Scale (modified)	Zinc vs. placebo ▪ 16 weeks gestation to birth	▪ – 1,2 ▪ Ø 3	14/21
Helland *et al.* (330, 331)	84 children (Norway)	1 (At 4 yrs)	Kaufman Assessment Battery for Children	Cod liver oil vs. corn oil ▪ 18 weeks gestation to three months post-birth	▪ + 1	12/21
Helland *et al.* (330, 331)	143 children (Norway)	1 (At 7 yrs)	Kaufman Assessment Battery for Children	Cod liver oil vs. corn oil ▪ 18 weeks gestation to three months post-birth	▪ Ø 1	15/21
Innis & Friesen (334, 335)	135 infants (British Columbia)	4 (At 60 d)	Teller Acuity Card Procedure	DHA vs. corn/soybean oil ▪ 16 weeks gestation to birth	▪ Ø 4	16/21

Reference	Sample	Outcomes	Measures	Intervention/Duration	Results	Quality Score
Joos *et al.* (340, 342)	99 infants (rural Taiwan)	1,2 (At 250 d)	Bayley Scales of Infant Development	HCHP drink + MVM vs. LCLP drink + MVM ▪ Preconception to lactation	▪ Ø 1 ▪ + 2	15/21
Judge *et al.* (332, 333)	30 infants (Connecticut)	4 (At 4 and 6 mo)	Teller Acuity Card Procedure	DHA vs. corn oil cereal bar ▪ 24 weeks gestation to birth	▪ + 4 at 4 months ▪ Ø 4 at 6 months	14/21
Judge *et al.* (332, 333)	29 infants (Connecticut)	1 (At 9 mo)	Willatts' Infant Planning Test[a] Fagan Test of Intelligence[b]	DHA vs. corn oil cereal bar ▪ 24 weeks gestation to birth	▪ + 1[a] ▪ Ø 1[b]	12/21
Li *et al.* (341)	1305 infants (rural China)	1,2 (At 3,6,12 mo)	Bayley Scales of Infant Development	MVM vs. folic acid + iron vs. folic acid alone ▪ 14 weeks gestation to birth	▪ + 1 at 12 months ▪ Ø 1 at 3 and 6 months ▪ Ø 2	19/21
Malcolm *et al.* (334, 335)	55 infants (Scotland)	4 (At 50 and 66 wk)	Visual evoked potential	Fish oil vs. sunflower oil ▪ 15 weeks gestation to birth	▪ Ø 4	15/21
McGrath *et al.* (340, 342)	327 children born to HIV-infected mothers (Tanzania)	1,2 (At 6, 12 and 18 mo)	Bayley Scales of Infant Development	Vitamin A vs. MVM-A vs. MVM+A vs. placebo ▪ < 28 weeks gestation to 18 months post-birth	▪ Ø 1 ▪ + 2 (MVMs only)	13/21
Parsons *et al.* (336)	264 children (Australia)	3 (At 7.5 yrs)	Strengths and Difficulties Questionnaire Short Temperament Scale for Children	Iron vs. placebo ▪ 20 weeks gestation to birth	▪ Ø 3	17/21
Schmidt *et al.*	276 infants	1,2	Bayley Scales of Infant	Iron + folic acid + vitamin A	▪ Ø 1, 2	14/21

Reference	Sample	Outcomes	Measures	Intervention/Duration	Results	Quality Score
(343)	(rural Indonesia)	(At 6 and 12 mo)	Development	vs. iron + folic acid vs. LD iron + LD folic acid ▪ 20 weeks gestation to birth		
Tamura *et al.* (328, 344)	355 children (Alabama)	1,2,4,5 (At 5.3 yrs)	Differential Ability Scales Wide Range Achievement Test Knox Cube test Gross Motor Scale Grooved Pegboard test Peabody Picture Vocabulary Test Visual Sequential Memory Auditory Sequential Memory	Zinc vs. placebo ▪ 20 weeks gestation to birth	▪ Ø 1,2,4,5	15/21
Tofail *et al.* (338)	249 infants (Bangladesh)	1,2,3 (At 10 mo)	Bayley Scales of Infant Development Wolke's Behaviour Rating Scale	Fish oil vs. soy oil ▪ 25 weeks gestation to birth	▪ Ø 1,2,3	15/21
Tofail *et al.* (336, 339, 345)	2116 infants (Bangladesh)	1,2 (At 7 mo)	Willatts' Infant Planning Test Bayley Scales of Infant Development – Psychomotor Developmental Index	Food packets + MVM vs. food packets + iron + folic acid (Fe60) vs. food packets + LD iron + folic acid (Fe30) ▪ < 30 weeks gestation to birth	▪ Ø 1,2	17/21
Zhou *et al.* (336,	302 children	1,3	Stanford-Binet Intelligence	Iron vs. placebo	▪ Ø 1,3	17/21

Reference	Sample	Outcomes	Measures	Intervention/Duration	Results	Quality Score
345)	(Australia)	(At 4 yrs)	Scale Strengths and Difficulties Questionnaire	▪ 20 weeks gestation to birth		

Key: 1= cognitive development, 2 = motor development, 3 = behavioral development, 4 = visual development, 5 = auditory development, Ø = no effect of nutrient(s) on development, + = positive effect of nutrient(s) on development, – = negative effect of nutrient(s) on development

MVM = multivitamin-mineral, HCHP = high calorie, high protein, LCLP = low calorie, low protein, MVM-A = multivitamin-mineral without vitamin A, MVM+A = multivitamin-mineral with vitamin A, LD = lower dosage

Table 14: Composition of micronutrients in studies included in the Systematic Review*

Study	Vitamin A (RAE)	Folic acid (mg)	Iron (mg)	Zinc (mg)	Iodine (mcg)	Copper (mg)	DHA (g)	EPA (g)
Caulfield *et al.* (328): "folic acid + iron"	-	250	60	-	-	-	-	-
Caulfield et al. (328): "folic acid + iron + zinc"	-	250	60	25	-	-	-	-
Dunstan *et al.* (346)	-	-	-	-	-		2.2	1.1
Hamadani *et al.* (337)	-	-	-	30	-	-	-	-
Helland *et al.* (330, 331)	1170	-	-	-	-	-	1.2	0.8
Innis & Friesen (334, 335)	-	-	-	-	-	-	0.4	-
Joos *et al.* (340, 342)	1500	-	12	-	-	1.0	-	-
Judge *et al.* (332, 333)	-	-	-	-	-	-	0.2	-
Li *et al.* (341): "MVM"	800	0.4	30	15	150	2.0	-	-
Li *et al.* (341): "iron + folic acid"	-	0.4	60	-	-	-	-	-
Li *et al.* (341): "folic acid"	-	0.4	-	-	-	-	-	-
Malcolm *et al.* (334, 335)	-	-	-	-	-	-	0.2	†
McGrath *et al.* (340, 342): "vitamin A"	6000	-	-	-	-	-	-	-
McGrath *et al.* (340, 342): "MVM-A"	-	0.8	-	-	-	-	-	-
McGrath *et al.* (340, 342): "MVM+A"	6000	0.8	-	-	-	-		-
Parsons *et al.* (336)	-	-	20	-	-	-	-	-
Schmidt *et al.* (343): "iron + folic acid + vitamin A"	4800	0.5	120	-	-	-	-	-
Schmidt *et al.* (343): "iron + folic acid"	-	0.5	120	-	-	-	-	-
Schmidt *et al.* (343): "LD iron + LD folic acid"	-	0.25	90	-	-	-	-	-
Tamura *et al.* (328, 344)	-	-	-	25	-	-	-	-

Study	Vitamin A (RAE)	Folic acid (mg)	Iron (mg)	Zinc (mg)	Iodine (mcg)	Copper (mg)	DHA (g)	EPA (g)
Tofail *et al.* (338)	-	-	-	-	-	-	1.2	1.8
Tofail *et al.* (336, 339, 345): "food + MVM"	800	0.4	30	15	150	2		
Tofail *et al.* (336, 339, 345): "food + Fe60"	-	0.4	60	-	-	-	-	-
Tofail *et al.* (336, 339, 345): "food + Fe30"	-	0.4	30	-	-	-	-	-
Zhou *et al.* (336, 345)	-	-	20	-	-	-	-	-

*A detailed description of other micronutrients in each study appears in Appendix 1.

† EPA content of intervention not specified. Per manufacturer information, EPA dosage was ~ 36 mg per day.

Table 15: Composition of (daily) micronutrient, macronutrient and fatty acid interventions in studies included in the systematic review

	Vitamin A (mcg RAE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (mcg)	Folic acid (mg)	Vitamin C (mg)	Vitamin D (IU)	Vitamin E (mg)	Iron (mg)	Zinc (mg)	Copper (mg)	Iodine (mcg)	Selenium (mcg)	DHA (g)	EPA (g)
Caulfield *et al*. (328) "zinc + iron + folic acid"	-	-	-	-	-	-	250	-	-	-	60	25	-	-	-	-	-
Caulfield et al. (328) "iron + folic acid"	-	-	-	-	-	-	250	-	-	-	60	-	-	-	-	-	-
Dunstan *et al.* (346)	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	2.2	1.1
Hamadani *et al.* (337)	-	-	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-
Helland *et al.* (330, 331)	1170	-	-	-	-	-	-	-	400	14	-	-	-	-	-	1.2	0.8
Innis & Friesen (334, 335)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-
[a,b]Joos *et al.* (340, 342) "HCHP drink"	1500	1.6	1.8	20.0	1.6	2.0	-	75	400	6.7	12	-	1.0	-	-	-	-
[b]Joos *et al.* (340, 342) "LCLP drink"	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	-
[c]Judge *et al.* (332, 333)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
Li *et al.* (341) "MVM"	800	1.4	1.4	18	1.9	2.6	0.4	70	200	10	30	15	2.0	150	65	-	-
Li *et al.* (341) "iron + folic acid"	-	-	-	-	-	-	0.4	-	-	-	60	-	-	-	-	-	-
Li *et al.* (341) "folic acid"	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-

	Vitamin A (mcg RAE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (mcg)	Folic acid (mg)	Vitamin C (mg)	Vitamin D (IU)	Vitamin E (mg)	Iron (mg)	Zinc (mg)	Copper (mg)	Iodine (mcg)	Selenium (mcg)	DHA (g)	EPA (g)
Malcolm *et al.* (334, 335)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
[d]McGrath *et al.* (340, 342) "vitamin A"	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
McGrath *et al.* (340, 342) "MVM-A"	-	20	20	100	25	50	0.8	500	-	30	-	-	-	-	-	-	-
[d]McGrath *et al.* (340, 342) "MVM+A"	6000	20	20	100	25	50	0.8	500	-	30	-	-	-	-	-	-	-
Parsons *et al.* (336)	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-
Schmidt *et al.* (343) "iron + folic acid + vitamin A"	4800	-	-	-	-	-	0.5	-	-	-	120	-	-	-	-	-	-
Schmidt *et al.* (343) "iron + folic acid"	-	-	-	-	-	-	0.5	-	-	-	120	-	-	-	-	-	-
Schmidt *et al.* (343) "LD iron + LD folic acid"	-	-	-	-	-	-	0.25	-	-	-	90	-	-	-	-	-	-
Tamura *et al.* (328, 344)	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-
Tofail *et al.* (338)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.8
[e]Tofail *et al.* (336, 339, 345) "food + MVM"	800	1.4	1.4	18	1.9	2.6	0.4	70	200	10	30	15	2	150	65	-	-

	Vitamin A (mcg RAE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (mcg)	Folic acid (mg)	Vitamin C (mg)	Vitamin D (IU)	Vitamin E (mg)	Iron (mg)	Zinc (mg)	Copper (mg)	Iodine (mcg)	Selenium (mcg)	DHA (g)	EPA (g)
[e]Tofail *et al.* (336, 339, 345) "food + Fe60"	-	-	-	-	-	-	0.4	-	-	-	60	-	-	-	-	-	-
[e]Tofail *et al.* (336, 339, 345) "food + Fe30"	-	-	-	-	-	-	0.4	-	-	-	30	-	-	-	-	-	-
Zhou *et al.* (336, 345)	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-

*****Nutrient content not quantified in study protocol

Additional compositions:

[a]Joos *et al*. (340, 342) – calcium 1000 mg, phosphorus 800 mg, sodium 400 mg, potassium 1.8 g, manganese 2 mg, fibre 1.1 g, 800 kcal, protein 40 g

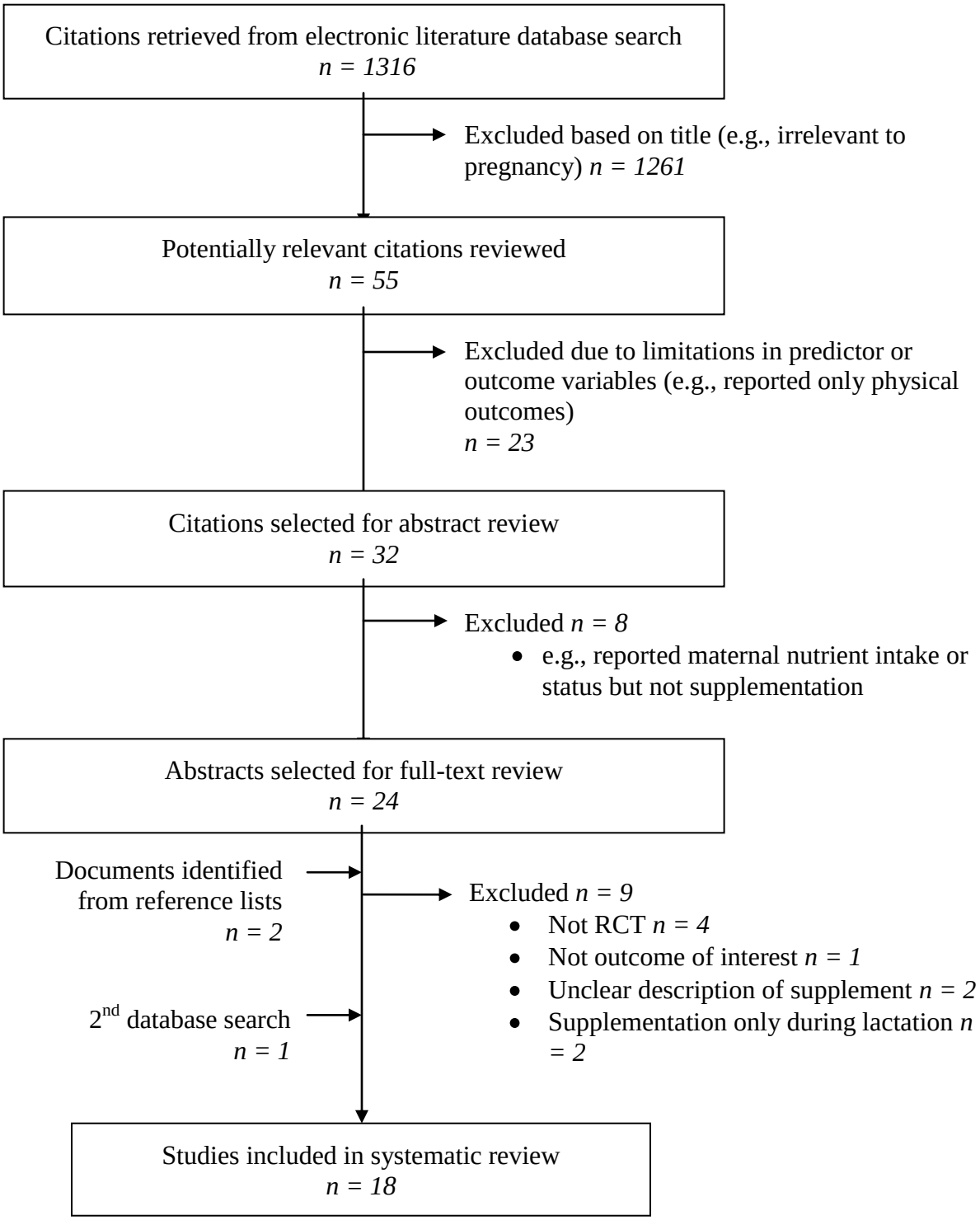
[b]Joos *et al*. (340, 342) – additional vitamins and minerals provided by multivitamin- mineral tablet were unspecified

[c]Judge *et al*. (332, 333) – 70 kcal, protein 1 g, carbohydrate 15 g

[d]McGrath *et al.* (340, 342) – vitamin A dose provided as 30 mg beta-carotene (4500 mcg RAE) and 5000 IU (1500 mcg RAE) preformed vitamin A

[e]Tofail *et al.* (336, 339, 345) – quantity of daily protein provided by food packets was unspecified

Figure 5: Selection of studies for systematic review of the effect of prenatal supplementation with micronutrients on infant/child development.



CHAPTER 8: DISCUSSION

Summary of main findings

Part 1 of the thesis examined the association between micronutrients and maternal mental health, while Part 2 explored the effect of maternal micronutrient supplementation on child neurocognitive development. The results from the global literature were mixed in regards to the protective properties of a number of nutrients (e.g. omega-3 fatty acids, folate, and iron) for maternal depression, while the results from the APrON study indicated that selenium supplementation was one of the multiple factors associated with reduced risk for postpartum depression. Furthermore, the APrON results showed that women who consumed the upper ranges for most micronutrients, and above the RDA for a number of micronutrients, were less likely to have depressive symptoms (e.g. having an EPDS score <10). While not conclusive, the evidence is growing for the potential benefit of micronutrient supplements for maternal mental health during pregnancy.

Studies about the impact of maternal supplementation on child neurodevelopment have also been inconsistent. The research literature indicated that supplementing with a single micronutrient likely has null effect on child neurodevelopment, while there was some evidence that maternal supplementation with multiple micronutrients may confer some benefits. Overall, the evidence was limited, and much more research would be required to draw any conclusion about the potential benefit of maternal supplementation on the developing foetus' brain, and consequently cognitive and behavioural health.

Challenges with studying nutrition and mental health

One of the biggest challenges to studying the effects of micronutrients on brain function (in this case, maternal mood and child behaviour) is the potential impact of both random and

systematic measurement errors often associated with dietary intake measures because of the complex nature of dietary intake assessment. As explained in Chapter 5, numerous efforts were made to ensure valid collection of supplement intake and minimize bias (recall, reporting, and measurement). However, also discussed in Chapter 5, in the case of multi-micronutrient supplementation, multi-collinearity continues to be problematic given that certain nutrients are commonly combined together in supplement form, thus it is a challenge to tease out the possible effect of one nutrient over another. This limitation was addressed with modeling and statistical techniques.

The nutrient intake discussed in the manuscripts was restricted to nutrients from supplements. Nutrient intake should not be equated with food intake, as foods consist of numerous substances in addition to nutrients (355). Nutrient intake is calculated from food or dietary intake, and the nutrients of interest are extrapolated from certain food types using standard food composition tables. Thus, nutrient intake through supplementation is *usually* included in assessments of dietary intake. Ordinarily, a number of factors must be considered in assessing nutrient intake: these include day to day (within person) variations of foods consumed, the method of assessment itself (i.e. how the dietary information is collected), and reporting errors (e.g. under or over reporting of specific foods and/or nutrients). However, in the case of nutrient intake derived from supplements only, we postulated that there would be less variation as the type/form of supplements being taken would be more consistent compared to the variation that existed with food intake.

Another limitation in assessing intake from supplementation is that the bio-availability of the micronutrient from supplements (quality and actual quantity) is usually unknown, as is the degree to which a nutrient is absorbed, metabolized, or utilized by the body (355). To address the

issue of absorption, laboratory measures would be helpful to assess blood levels of the nutrients. However, as stated in Chapter 5, values from blood samples from the APrON study were not available at the time of the analysis. Appendix 8 provides details on Nutrient Assessment, including how the Dietary Reference Intakes (DRI) values are derived and how measurements from studies on intake are assessed, Table 16 gives the definition for the DRIs, and Tables 17a and 17b provide the values of the various DRIs, as well as the various methods used to measure dietary intakes, from food frequency questionnaires, to 24 hour food recall, to 3 day food records.

In the absence of data on serum levels, some issues cannot be addressed in this thesis, but two issues arising from the research require further discussion: 1) supplementing with single versus multiple micronutrients, and 2) the intake of excess amounts of some micronutrients.

Single versus multiple micronutrient supplementation

There is some evidence that suggests single and multiple micronutrient supplements have different impacts on maternal and foetal health. A project that also examined the effects of prenatal multi-micronutrients on maternal mood and on child cognition was the SUMMIT (Supplementation with Multiple Micronutrients Intervention Trail) Study Group, a double-blind cluster-randomized trial in Indonesia (356). The trial compared pregnant women supplemented with multi-micronutrients to those receiving only iron + folic acid. Maternal mood (n = 640) was assessed in an interview format with a scale derived from the Centre for Epidemiological Studies Depression Scale (356). The researchers found that women supplemented with multi-micronutrients had similar mood scores to those supplemented with just iron + folic acid (356). However, women supplemented with multi-micronutrients had a benefit of 0.12 SD on overall cognition, compared to those taking only iron + folic acid (95% CI 0.03-0.22, p = .010), and a

benefit of 0.18 SD on reading efficiency (95% CI 0.02-0.35, p = .031), after controlling for maternal age, education, and socio-economic status (356). The researchers concluded that the improvement in cognition was not dependent on enhanced mood (356).

Another analysis of the data from SUMMIT assessed child cognition as a function of maternal supplementation. The children were tested at age 3 and a half years on cognitive development, including language ability, visuospatial ability, visual attention, and executive function (n = 487) (357). No difference was found in cognitive development between children of mothers who received multi-micronutrient supplements compared to mothers who took iron + folic acid only (357). However, when stratified on maternal nourishment status (as measured by mid-upper arm circumference and anemia at enrolment), children of undernourished mothers who received the multi-micronutrients scored higher on the visual attention/spatial ability tests than those who took iron + folic acid, $\beta = 0.37$ (95% CI: 0.11 to 0.62); P = .004 for children of malnourished mothers with mid-upper arm circumference <23.5 cm), and $\beta = 0.24$ (95% CI: 0.02 to 0.46); P = .030 for children of anaemic mothers (hemoglobin concentration <110 g/L) (357). The study concluded that prenatal multi-micronutrients were more beneficial in improving cognitive and motor development of children of undernourished or anaemic women than iron + folic acid alone (357). It further demonstrated that adequate nutrient status in the mother is necessary for proper brain development in the foetus.

A recent systematic review of multi-micronutrient supplementation for the treatment of psychiatric symptoms demonstrates mixed results (222). While there was some evidence in the treatment of stress, antisocial behaviours, and depressed mood (mainly in the nonclinical and elderly populations), there was less data to support the treatment of bipolar disorder, ADHD, and substance abuse/dependence with multi-micronutrients (222). Other studies compared the effect

of multi-nutrients versus iron and folic acid focused on birth outcomes. A review of clinical trials registered with the Cochrane Pregnancy and Childbirth Group by Haider and Bhutta reported women who took multi-micronutrients had statistically significant decreases in the number of low birth weight babies (RR = 0.83; 95% CI: 0.76 to 0.91), small-for-gestational-age babies (RR = 0.92; 95% CI: 0.86 to 0.99) and in maternal anemia (RR = 0.61; 95% CI: 0.52 to 0.71). But the differences were not statistically significant when compared with iron + folic acid supplementation alone (358). In the APrON study, almost all women took multi-micronutrients, with few (< 1%) taking a single micronutrient, such as folic acid, iron, or omega 3. Thus, there was insufficient APrON data on the intake of single supplements for comparison purposes. Based on the literature, and the findings from the APrON study, the effect of single versus multi-micronutrient supplementation needs further investigation.

Excess micronutrient intake

The trend of consuming micronutrients through supplements is on the rise. Consumers may not be aware of the amount of micronutrients being ingested, as they are coming from multiple sources. Excess intake in this thesis is defined as the amount above the Tolerable Upper Intake Levels (UL). The UL of the DRI is the highest amount of a nutrient consumed daily that is unlikely to cause symptoms in most people in the general population. The UL has been determined for a number of nutrients (see Table 17a).

While the main concern worldwide has been nutrient insufficiency, in some developed countries, such as Canada, there is growing concern about the effects of excess intake. It has been proposed that the risk and benefit from micronutrients can be extracted from a dose-response curve, and that there is likely to be inter-individual variations in response to the continuum; that is, the level which is toxic to one individual may be tolerated by another (359).

As well, different nutrients would have different dose-response ranges, where too low intake results in deficiency, and too high intake leads to toxicity (360). Mulholland and Benford also proposed a sequence of adverse effects with increasing order of severity, from minor biochemical changes without clinical presentation, to reversible clinical features, to severe clinical features and irreversible organ damage (359). For example, large doses of vitamin C or magnesium have been associated with diarrhea, while iron has resulted in constipation, with nausea, vomiting, and epigastric pain (359), all of which are symptoms of no great clinical concern and easily reversible. In contrast, large doses of vitamin A in the form of retinol have led to congenital abnormalities in the offspring (359). The adverse effect may be associated with a specific population, as in the case of the intervention trial that found B-carotene was associated with an increase in the incidence of lung cancer specifically in people who were smokers (359). The following are nutrients with known adverse effects when taken in excess (360) include:

- Vitamin A (retinol) → liver damage, congenital abnormalities, increased risk of hip fracture
- Vitamin D (calciferol) → hypercalcaemia, weakness
- Vitamin E (tocopherol) → decreased blood coagulation in patients taking anti-coagulant drugs
- Vitamin B6 → neurotoxicity
- Zinc → impaired copper absorption
- Selenium → Selenosis (gastrointestinal disorder, hair loss, sloughing of nails, fatigue, neurological damage)
- Iodine → thyroid hyperactivity

There are many nutrients whose ULs have yet to be determined, and for which harmful intakes have not been reported, even at high doses: e.g., vitamins B12, B5, B1, biotin, manganese (360).

An analysis of multi-micronutrient supplementation in the Hawaii-Los Angeles Multiethnic Cohort reported that of the 48% of men and 56% of women without chronic diseases who reported taking multivitamin supplements regularly, potentially excessive intakes were most likely for iron, zinc, vitamin A, and niacin (361). A review of clinical trials from the Cochrane Registry by Haider and Bhutta found insufficient data from the studies they included in their analysis to examine the adverse effects of multiple micronutrient supplementation in pregnant women (358).

In the APrON cohort, a proportion of pregnant women were taking some micronutrients above the UL, in particular, folic acid (85.9%), vitamin D (18.5%), and iron (17.6%) (see Table 18). While the adverse effect of excess iron and vitamin D intake on individual health has been documented, that is not the case for folic acid. The short and long term impact of high levels of these three nutrients on foetal health in humans has yet to be determined. However, animal models have provided interesting data on the excess intake of these micronutrients.

Excess Folic acid

As presented in the MES report, a majority of pregnant women reported taking folic acid supplements before (57.7%) and during (89.7%) pregnancy (23). While the MES did not provide the dose and frequency, it is clear that the amount of folic acid would be additional to the intake of folate from food sources (including fortified foods), thus greatly increasing their regular intake from diet alone. The primary concerns related to excess folate/folic acid intake in the general population are threefold (362):

1. masking the effects of pernicious anemia resulting in neurologic deterioration due to vitamin B12 deficiency at amounts of 5 mg or more folic acid/day;
2. disrupting zinc function; however, results of different studies have been inconsistent, and clinical trials have found no adverse effect in the pregnant population due to zinc function disruption or other mechanisms;
3. interfering with medications such as anticonvulsants (e.g. diphylhydantoin) at higher doses of 5 to 30 mg (no evidence of effect at lower intakes of folic acid), and methotrexate (a folate antagonist).

Review of studies on these three potential adverse effects of excess folic acid showed mixed results; that is, no consistent outcome associated with the excess intake of folic acid (362). There is evidence from animal studies that excess folic acid intake may have adverse effects on the fetus. A study by Mikael and colleagues (364) found pregnant rats fed a diet supplemented with a 10-fold higher amount of folic acid (20 mg/kg diet) had more embryonic loss, embryonic delays, a higher incidence of ventricular septal defects, and thinner left and right ventricular walls in the embryo, compared to mothers fed a control diet (2 mg/kg diet). They concluded that moderately high levels of folic acid supplementation may adversely affect fetal mouse development, and thus studies are warranted to evaluate the impact of high folate intake in pregnant women (364). In another animal study by Pickell and colleagues (365), pregnant rats fed a diet supplemented with folic acid 20-fold higher than the recommended intake, had embryonic delay and growth retardation, and apparently greater susceptibility to embryonic defects (e.g. thinner ventricular walls in embryonic hearts), but no adverse affect on placental development. However, a study investigated of chromosome damage in mice reported that excess folic acid (6 mg folic acid/kg diet) did not appear to promote or protect against

chromosome damage (366). The results suggested that chronic exposure to folic acid at the levels similar to those achieved through fortification in the human diet was unlikely the cause chromosome damage (366). Thus, even in animal studies, the results have been mixed regarding the adverse effects of excess folic acid intake.

Results from human studies did not follow those from animal studies. A recent study by Papadopoulou and colleagues (363) in a subsample (n = 58) of the mother-child cohort in Crete, Greece (Rhea study) examined whether high doses of folic acid and iron supplementation in early-to-mid pregnancy affect the risk of preterm birth, low birth weight, and small for gestational age neonates. They found 66% reported high doses of supplemental folic acid use (5 mg/day), while 21% reported excessive doses of folic acid use (>5 mg/day) in early-to-mid pregnancy. Daily intake of 5-mg supplemental folic acid was associated with a decrease in the risk of preterm birth (by 31%), low birth weight neonate (by 60%), and delivering a small for gestational age (SGA) neonate (by 66%) (363). In contrast, daily doses of iron supplementation more than 100 mg were associated with a twofold increased risk for SGA neonates (363). They concluded that high daily doses of supplementary folic acid in early-to-mid pregnancy may be protective for preterm birth, low birth weight, and small for gestational age neonates, while high daily doses of supplementary iron may be harmful for fetal growth (363).

Excess Vitamin D

Vitamin D toxicity may result in hypercalcemia (fatigue, depression, anorexia, nausea, constipation, polyuria, polydipsia, or muscle weakness) or hypocalcemia (paresthesias of the lips or extremities, muscle spasm, carpopedal spasm, seizures, weakness, or abdominal cramping). Cholecalciferol (D3) is the natural form of vitamin D, while ergocalciferol (D2) is the synthetic form. Both forms have equivalent absorption efficiency, but D2 is less potent, and thus has a

different toxicity profile (367). Toxicity doses recorded in the general population for D3 ranged from 400 IU (one aberrant case in an 85 year old woman) to 600,000 IU (isolated incident) from case reports, with hypercalcemia as the main outcome (367). In clinical trials in healthy adults, doses up to 10,000 IU vitamin D3 have been tested with no reports of toxicity. A review by Heaney found toxicity was associated with supplement intake in the amount well above 20,000 IU/day (368). No adverse events in the pregnant population have been documented, or the impact of high maternal intake on the mother or foetus has been reported (367).

Some relevant evidence on the adverse effects of very excessive vitamin D intake from supplementation has come from case reports. Stephenson and Peiris described a 56-year-old woman who took mega doses of ergocalciferol (D2) (150,000 IU orally daily) for 28 years and did not show any signs of toxicity (369). They proposed that a possible mechanism that may account for the lack of toxicity was the reduced efficacy of ergocalciferol compared to cholecalciferol (D3) combined with the lack of significant calcium supplementation (369). In another case report, Kaptein and colleagues reported on two women, aged 75 and 73, who had life-threatening hypercalcemia as a result of vitamin D3 intake that was a 100–1,000 times higher than stated on the label of 150 IU (370). The main difference in this case was that the patients took D3 instead of D2, which appears to confer greater toxicity. The effect of excess vitamin D intake on maternal mood and child development remains unknown, and further study is required.

Human data on the adverse effects from excess vitamin D intake has been limited, but studies from various animal models shed some insight into possible effects. The Expert Group on Vitamins & Minerals of the Food Standard Agency of the UK presented findings from animal studies in their report (371). One study found excess vitamin D2 during gestation in rabbits

resulted in a decrease in fetal viability, increase in the number of spontaneous abortions, and increase in the cases of supravalvular lesions in the offspring. In a study of rodents, high levels of vitamin D2 during gestation resulted in retarded fetal and placental growth, loss of ossification of fetal bones, and fetal skeletal degeneration (371). Capen and colleagues (372) found that hypervitaminosis D in pregnant cows caused more severe lesions in cardiovascular tissues, compared to non-pregnant cows. A review by Jones (373) of animal studies (including rats, cows, pigs, rabbits, dogs, and horses) on vitamin D intoxication, as measured by plasma D3 metabolite (25(OH)D3) concentrations, found the outcome of hypercalcemia to be consistent in all the animals studied. However, Jones did not report findings from studies of vitamin D excess in pregnant animals (373).

Excess Iron

Prolonged excess iron (i.e. iron overload) intake may resemble a genetic condition called hemachromatosis, where abnormal absorption from the duodenum and upper intestine results in deposits accumulating in the liver, pancreas, heart, pituitary gland and skin, and consequently causes damage to those organs due to oxidative stress generated by the excess iron (374). Iron overload has been associated with increased infection, neoplasia, cardiomyopathy, atherosclerosis, and chronic liver disease and hypertension (374, 375).

So far, the evidence on the adverse effects of high iron concentrations has been derived from *in-vitro* and epidemiological studies, thus it has been difficult to obtain data from reproducible cause-effect and dose-response relationship for adverse health effects that suggest a relationship to iron intake (375). Furthermore, the evidence on excess iron intake comes from the non-pregnant population. In the pregnant population, the use of oral iron to prevent maternal anemia is well established (376). The debate continues with regard to the appropriate dose when

supplementing during pregnancy (376). According to Milman (376), in pregnant women, iron supplementation may,

(i) increase oxidative stress locally in the small intestines,

(ii) increase oxidative stress in the body in general,

(iii) cause gastrointestinal side effects at high iron doses,

(iv) accelerate body iron overload in women with (nondiagnosed) genetic hemochromatosis

An adequate supply of iron is essential for normal development of the foetus and newborn child, in particular for foetal brain and cognitive abilities of the newborn (376). However, a review by Rao and Georgieff reported full-term infants with high cord serum ferritin concentrations were more likely to have lower IQ at age 5 (377). In this case, it is unclear if the increased ferritin concentration was a result of foetal iron overload, as protein-bound iron in the form of ferritin has not been associated with adverse effects (377). Furthermore, it is unknown whether enteral iron supplementation could result in oxidative stress during the perinatal period. Iron supplementation in doses as high as 12 μg/kg per day has not been associated with oxidative stress in infants (377). The evidence of excess iron intake on maternal mood and child development is very limited.

The Expert Group on Vitamins & Minerals of Food Standard Agency in the UK reviewed the literature and reported on the evidence of adverse effects of iron from human and animal studies (371). They found that studies from animal models were limited. For example, a multi-generational study in rats found no adverse effect of excess maternal iron (20 mg/kg by weight/week, given intramuscularly) on offspring growth or weight; as well, there was no evidence that excess iron transferred across the placenta (371). Another study of maternal iron

overload in a bovine model reported no corresponding elevation of fetal serum iron levels despite extremely elevated maternal serum iron concentration (371). One study reported that supplements of iron gluconate (administered intraperitoneally) given to pregnant mice resulted encephalopathy, but the results have not been duplicated (371). A study by Park and colleagues (378) investigated the effect of maternal excess iron intake on oxidative stress in the placenta during pregnancy. Pregnant dams were fed a diet with normal iron (35mg/kg diet), high iron (350mg/kg diet) or excess iron (1050mg/kg diet) during pregnancy. The results showed liver iron and ferritin of pregnant dams were significantly increased with increasing amounts of maternal iron intake (378). Also, iron and ferritin levels in the placenta were more increased in the high and excess iron groups than normal. They also reported increase of oxidation markers but a decrease in antioxidant enzyme (e.g. glutathione peroxidase and catalase) activity in placentas in the excess Fe group (378). The authors concluded that excess maternal iron intake may increase oxidative stress of the placenta, and postulated that it could affect redox status of fetal and neonatal tissue adversely (378). Fredriksson and colleagues (379) administered iron succinate (7.5 mg/kg) versus saline postnatally to mice. They found behavioural deficits, as well as maze learning and motor activity deficits in animals treated with iron, most significant in those exposed during postnatal days 3–5 (379). Another study by Fredriksson and colleagues (380) investigated the long-term neurobehavioral effects of iron exposure by treating mice orally with 0.0, 3.7, or 37.0 mg iron/kg body weight postnatally. The results showed mice treated with the higher dose of iron, 37.0 mg/kg, were hypoactive during the first 20 minute of testing but hyperactive during the final 20 minute, indicating a lack of habituation of spontaneous activity (380). These changes corresponded to a dose-response relationship. In the radial arm maze, the iron 37.0 mg/kg group had significantly more errors and longer latencies in behaviour in radial

arm maze learning (380). Analysis of brain iron content indicated significantly more total iron in the basal ganglia, but not the frontal cortex, of the higher, 37 mg/kg, dose group (380). The authors concluded that while knowledge of the impact of iron during the critical period of rapid brain growth is limited, increased iron levels in the brain, especially in the basal ganglia, may contribute to neurodegenerative processes (380).

Implications for public health and clinical practice

The evidence presented in this thesis provides further support for the idea that pregnant women and their children may benefit from the intake of multi-micronutrient supplementation. This evidence is in accordance with current practice, as pregnant women are taking supplements. However, the amounts and types taken are not often monitored by their healthcare practitioners. Few studies have documented the pattern and level of supplement intake. Given excess micronutrient intakes are a recent phenomenon, potential (adverse) effects and long term consequences are unknown at present. However there is potential for adverse effects in the short term (e.g. for the mother), and in the long term for the fetus, that needs close monitoring with regular clinical follow up between a woman and her doctor. Furthermore, a formal surveillance system may be beneficial to monitoring the amount of supplement intake, potential adverse reactions, and/or interactions with foods/medications.

Directions for future research

Causality cannot be established with observational studies, such as cohort and case control studies. In some cases, we may establish a strong association between nutrient(s) and the outcome of mood or behaviour. In the APrON study, data was collected prospectively, and strengthened the associations reported. However, lab values to measure serum levels would help to determine the amount of key nutrients and provide a clearer picture of the association. As

well, since it is unknown how well micronutrients from supplements are absorbed by the mother, and how much is transferred to the foetus, laboratory values for nutrient status would be helpful. Laboratory assays of nutrient status would also help to assess for specific nutrients that may be beneficial for the treatment of ASD, so that in the future, physicians may customize a treatment plan using multi-micronutrient supplementation.

Review of the global literature regarding the association of micronutrient supplements with 1) maternal mood, and 2) child neurocognitive development revealed that study design and methods were often poor; that is, there were inconsistent measurement tools used, and outcomes evaluated. In some cases, sample sizes were too small, follow up was too short, dosage was too low, intervention was inadequately recorded, and there was inadequate control for confounders in the analyses, making pooling of results difficult. Thus, future research is warranted to address these deficiencies.

Building on the current findings, as well as the literature reviewed in this thesis, some interesting questions that need to be addressed by future research include:

- Is there a difference in depressive symptoms in pregnant women supplemented with selenium alone compared to using selenium in a multi-nutrient complex?
- How well are intake levels reflected in serum or plasma concentration (status) for various micronutrients?
- What is the (long term) impact of excess micronutrient intake on maternal mental health (e.g. mood)?
- What is the (long term) impact of excess perinatal micronutrient intake on the neurocognitive development of foetus?

The evidence is growing that multi-micronutrients may confer benefits to mental health in pregnant women and to neurocognitive development. The results presented here, together with those of other studies, strongly indicate that further research is needed to build knowledge that may change practice in clinical and public health.

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Appendix 1: Edinburgh Postnatal Depression Scale

As you are pregnant, we would like to know how you are feeling. Please check the answer that comes closest to how you have felt **IN THE PAST 7 DAYS**, not just how you feel today.

In the past 7 days:

1. I have been able to laugh or see the funny side of things	☐ As much as I always could	☐ Not quite so much now
	☐ Definitely not so much now	☐ Not at all
2. I have looked forward with enjoyment to things	☐ As much as I ever did	☐ Rather less than I used to
	☐ Definitely less than I used to	☐ Hardly at all
3. I have blamed myself unnecessarily when things went wrong	☐ Yes, most of the time	☐ Yes, some of the time
	☐ Not very often	☐ No, never
4. I have been anxious or worried for no good reason	☐ Not at all	☐ Hardly ever
	☐ Yes, sometimes	☐ Yes, very often
5. I have felt scared or panicky for no very good reason	☐ Yes, quite a lot	☐ Yes, sometimes
	☐ No, not much	☐ No, not at all
6. Things have been getting on top of me (feeling overwhelmed)	☐ Yes, most of the time I haven't been able to cope at all	☐ Yes, sometimes I haven't been coping as well as usual
	☐ No, most of the time I have coped quite well	☐ No, I have been coping as well as ever
7. I have been so unhappy that I have had difficulty sleeping	☐ Yes, most of the time	☐ Yes, sometimes
	☐ No, not very often	☐ No, not at all
8. I have felt sad or miserable	☐ Yes, most of the time	☐ Yes quite often
	☐ No, not very often	☐ No, not at all
9. I have been so unhappy that I have been crying	☐ Yes, most of the time	☐ Yes quite often
	☐ No, only occasionally	☐ No, never
10. The thought of harming myself has occurred to me	☐ Yes, quite often	☐ Sometimes
	☐ Hardly ever	☐ Never

Appendix 2: Symptom Checklist-90-Revised Anxiety Dimension Scale

Below is a list of problems people sometimes have. Please read each one carefully, and check the answer that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU DURING THE PAST 7 DAYS INCLUDING TODAY. Check the answer for each problem and do not skip any items. If you change your mind, erase your first mark carefully.

In the Past 7 Days

Not at all	A little bit	Moderately	Quite a bit	Extremely	How much were you distressed by:
☐	☐	☐	☐	☐	Nervousness or shakiness inside
☐	☐	☐	☐	☐	Feeling critical of others
☐	☐	☐	☐	☐	Trouble remembering things
☐	☐	☐	☐	☐	Feeling low in energy or slowed down
☐	☐	☐	☐	☐	Trembling
☐	☐	☐	☐	☐	Feeling that most people cannot be trusted
☐	☐	☐	☐	☐	Suddenly scared for no reason
☐	☐	☐	☐	☐	Temper outbursts that you could not control
☐	☐	☐	☐	☐	Blaming yourself for things
☐	☐	☐	☐	☐	Feeling fearful
☐	☐	☐	☐	☐	Feeling others do not understand you or are unsympathetic
☐	☐	☐	☐	☐	Having to do things very slowly to ensure correctness
☐	☐	☐	☐	☐	Heart pounding or racing
☐	☐	☐	☐	☐	Feeling inferior to others
☐	☐	☐	☐	☐	Trouble concentrating
☐	☐	☐	☐	☐	Feeling tense or keyed up
☐	☐	☐	☐	☐	Heavy feelings in your arms or legs
☐	☐	☐	☐	☐	Feeling uneasy when people are watching or talking about you
☐	☐	☐	☐	☐	Spells of terror or panic
☐	☐	☐	☐	☐	Getting into frequent arguments
☐	☐	☐	☐	☐	Feeling so restless you couldn't sit still
☐	☐	☐	☐	☐	Feelings of worthlessness
☐	☐	☐	☐	☐	The feeling that something bad is going to happen to you
☐	☐	☐	☐	☐	Shouting or throwing things
☐	☐	☐	☐	☐	Thoughts and Images of a frightening nature

Appendix 3: Modified National Population Health Survey – Social Support

Now we would like to ask you a few questions about your support from family, friends, and the community.

1. Do you have someone you can confide in, or talk to, about your private feelings or concerns?	☐ none of the time	☐ a little of the time	☐ about half of the time	☐ most of the time	☐ all of the time
2. Do you have someone you can really count on to help you in a difficult situation?	☐ none of the time	☐ a little of the time	☐ about half of the time	☐ most of the time	☐ all of the time
3. Do you have someone you can really count on to give you good advice when you are making important personal decisions?	☐ none of the time	☐ a little of the time	☐ about half of the time	☐ most of the time	☐ all of the time
4. Do you have someone who makes you feel loved and cared for?	☐ none of the time	☐ a little of the time	☐ about half of the time	☐ most of the time	☐ all of the time

Appendix 4: Modified Stressful Life Events Questionnaire

A. Recent Life Events During this Pregnancy

Listed below are a number of events that may have brought changes in your life. Please tell us if any of these events happened to you in this pregnancy. If yes, please assess how much effect it had on you. Your answers will be held in strict confidence. ***Check all that apply.***

	No this has never happened *during this pregnancy.*	**Yes this has happened *during this pregnancy***	**If yes, how much did this affect you?**		
			Not at all	**Somewhat**	**A lot**
1. A close friend/family member had a serious accident/illness	☐	☐ →	☐	☐	☐
2. You were separated/divorced	☐	☐ →	☐	☐	☐
3. A close friend or relative died	☐	☐ →	☐	☐	☐
4. You had a serious argument with your partner	☐	☐ →	☐	☐	☐
5. Your partner was emotionally cruel to you	☐	☐ →	☐	☐	☐
***6.* Your partner was physically cruel to you**	☐	☐ →	☐	☐	☐
7. You were sexually abused	☐	☐ →	☐	☐	☐

B. Life Events 12 months before this pregnancy

In the 12 months before this pregnancy, had any of these events happened to you? Many of the events listed are of a personal nature. Your answers will be held in strict confidence. **Check all that apply.**

	No this has never happened *in the 12 months before this pregnancy.*	Yes this has happened *in the 12 months before this pregnancy*	If yes, how much did this affect you?		
			Not at all	Somewhat	A lot
1. A close friend/family member had a serious accident/illness	☐	☐ →	☐	☐	☐
2. You were separated/divorced	☐	☐ →	☐	☐	☐
3. A close friend or relative died	☐	☐ →	☐	☐	☐
4. You had a serious argument with your partner	☐	☐ →	☐	☐	☐
5. Your partner was emotionally cruel to you	☐	☐ →	☐	☐	☐
6. Your partner was physically cruel to you	☐	☐ →	☐	☐	☐
7. You were sexually abused	☐	☐ →	☐	☐	☐

C. Past Life Events

Before the age of 17, have any of these events happened to you? Many of the events listed are of a personal nature. Your answers will be held in strict confidence. **Check all that apply.**

	No, this never happened before the age of 17	Yes, this happened before the age of 17	If yes, how much did this affect you?		
			Not at all	Somewhat	A lot
1.A close Friend/family member had a serious accident/illness	☐	☐ →	☐	☐	☐
2. A close friend or relative died	☐	☐ →	☐	☐	☐
3. You were sexually abused	☐	☐ →	☐	☐	☐

Appendix 5: Personal Health History Questionnaire

In the following table, check "no" or "yes" to tell us if you currently have each medical condition. If you have a medical condition, please write any prescription medications you currently take for the condition, or prescription medications that you stopped taking for the condition because of pregnancy.

	Do you currently have this condition?		**If YES** Medications you currently take for this condition	**If YES** Medications for this condition you stopped because of pregnancy
	NO	**YES**		
Anxiety	☐ no	☐ yes---->	_____	_____
Asthma	☐ no	☐ yes---->	_____	_____
Celiac	☐ no	☐ yes---->	_____	_____
Depression	☐ no	☐ yes---->	_____	_____
Diabetes: Type 1 (insulin dependent)	☐ no	☐ yes---->	_____	_____
Diabetes: Type 2 (non insulin dependent)	☐ no	☐ yes---->	_____	_____
Diabetes: Gestational	☐ no	☐ yes---->	_____	_____
Epilepsy (seizures)	☐ no	☐ yes---->	_____	_____
Heart disease	☐ no	☐ yes---->	_____	_____
Primary (chronic) hypertension (you had high blood pressure before pregnancy)	☐ no	☐ yes---->	_____	_____
Gestational hypertension (high blood pressure that developed about half way through your pregnancy)	☐ no	☐ yes---->	_____	_____

	Do you currently have this condition?		If YES Medications you currently take for this condition	If YES Medications for this condition you stopped because of pregnancy
	NO	YES		
Pre-eclampsia (toxemia): high blood pressure caused by symptoms like protein in urine and possibly changes in blood and the liver.	☐ no	☐ yes---->		
Hypothyroidism (low functioning thyroid)	☐ no	☐ yes---->		
Hyperthyroidism (overactive thyroid)	☐ no	☐ yes---->		
Inflammatory bowel disease: ulcerative colitis	☐ no	☐ yes---->		
Inflammatory bowel disease: Crohn's disease	☐ no	☐ yes---->		
Irritable bowel syndrome	☐ no	☐ yes---->		
Polycystic Ovary Syndrome (PCOS)	☐ no	☐ yes---->		
Other current medical conditions ?	☐ no	☐ yes **(specify below)**		
1.				
2.				
3.				
4.				
5.				

Appendix 6: Socio-demographic & Background Information Questionnaire

1. What is your marital status? At present are you:

- ☐ Single (NEVER MARRIED)
- ☐ Married
- ☐ Divorced
- ☐ Common-law/living with partner/ living as married
- ☐ Widowed
- ☐ Separated

2. What is the highest level of education that you have completed?

- ☐ Less than high school diploma
- ☐ Completed high school diploma
- ☐ Completed trade, technic or vocational school, or business/ community college (e.g., SAIT, NAIT, Devry)
- ☐ Completed university undergraduate degree
- ☐ Completed post-graduate degree

3. What is the total income, **before taxes and deductions,** of all the household members from all sources in the past 12 months? (**YOUR BEST GUESS IS OK**)?

- ☐ Less than $20, 0000
- ☐ $20,000-$39,999
- ☐ $40,000-$69,999
- ☐ $70,000-$99,999
- ☐ $100,000 or more

4. What is your *partner's* occupation? (**PLEASE BE SPECIFIC**)

_____

- ☐ I do not have a partner

5. How many adults (over the age of 18) live in your household?

☐☐

6. How many children (under the age of 18) live in your household (**NOT INCLUDING THIS PREGNANCY**)? ☐☐

6. a. If children live in your household, what are their ages? (**START WITH THE FIRST BORN CHILD**)

1. ☐☐ Years ☐☐ Months
2. ☐☐ Years ☐☐ Months
3. ☐☐ Years ☐☐ Months
4. ☐☐ Years ☐☐ Months
5. ☐☐ Years ☐☐ Months
6. ☐☐ Years ☐☐ Months

If more than 6 children live in your household write their ages below: _____

7. How many people smoke **inside** your home? (**DO NOT INCLUDE THEM IF THEY SMOKE ONLY OUTSIDE**)

☐☐ number of people

8. Were you born in Canada?

☐ Yes ☐ No

(If no) how long have you lived in Canada?

☐ Less than 1 year ☐ 1 to 3 years ☐ 4 to 5 years ☐ >5 years

9. How would you **best** describe your ethnic origin (race)?

☐ Black (African, African North American)
☐ Caucasian/white (e.g. English, French, German, Greek, Irish, Polish, Russian, Scottish, Ukrainian)
☐ Chinese
☐ Filipino
☐ Japanese
☐ Korean
☐ Latin American
☐ Native/Aboriginal Peoples of North America (First Nations, North American Indian, Metis, Inuit)
☐ South Asian
☐ South East Asian
☐ Arab
☐ West Asian

Other, specify_____

Appendix 7: Supplement Intake Questionnaire

Participant ID:
Initials:
Interviewer:

SECTION I. DIETARY SUPPLEMENTS

Instructions: The following questions are about the use of dietary supplements (vitamins, minerals, herbal / botanical remedies, teas, homeopathic medicines and animal derived products).[1]

1. Are you currently using or taking any **vitamins, minerals or other dietary supplements**? Include those products prescribed by a health professional and those that do not require a prescription.

- ❑ YES
- ❑ NO – Continue to Section II.
- ❑ Did not know – Continue to Section II.

2. When did you begin using dietary supplements?

- ❑ Before pregnancy. How long before? _____
- ❑ During pregnancy. Which trimester? _____

3. From the list below (show participant the list), please tell me **all** of the following **supplements** that you are currently taking.

Dietary Supplement	**1st Visit** **Time Point:** **Date:**		**2nd Visit** **Time Point:** **Date:**		**3rd Visit** **Time Point:** **Date:**		**4th Visit** **Time Point:** **Date:**	
Multivitamins:	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency
Biquest Prenatal Multivitamins								
Centrum Materna								
Compliments Prenatal Multivitamins								
Equate Prenatal Multivitamins								
Exact Prenatal Vitamin and Mineral Supplement								
Generic Prenatal Multivitamins								
Health Balance Prenatal Multivitamins								
Kirkland Prenatal Multivitamins								

Dietary Supplement	1st Visit Time Point:		2nd Visit Time Point:		3rd Visit Time Point:		4th Visit Time Point:	
Multivitamins:	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency
Life Brand Prenatal Multivitamins								
London Drugs Prenatal and Postpartum Multivitamins								
Materna								
Materna Me								
"No Name" Brand Prenatal Multivitamins								
Nutralife™ Multiple Vitamins And Minerals For Prenatal Women								
One a Day Women's Prenatal								
PregVit								
PregVit Folic 5								
President's Choice Prenatal Multivitamins								
SISU - Multi Expecting								
Western Family Prenatal Multivitamins								
Other (please specify brand and/or DIN / NHPN):								

Comments:

Dietary Supplement	1st Visit Time Point:		2nd Visit Time Point:		3rd Visit Time Point:		4th Visit Time Point:	
Single supplements:	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency	✓	Dose / Frequency
B6 / Pyridoxine								
B-Complex								
Beta-Carotene								
Calcium / Calcium Supplements								
Coenzyme Q								
Compliments – Folic Acid								
Compliments – Vitamin D								
Fibre / Fiber Supplements								
Folic Acid / Folate								
Glucosamine								
Hydroxytryptophan (HTP)								
Iron								
Life Brand Prenatal Omega 3								
Life Brand Iron supplement								
Life Brand “Psyllum Husk” (fiber supplement)								
Niacin								
Phosphorous								
Selenium								
Vitamin A								
Vitamin C								
Vitamin D								
Vitamin E								
Zinc								
Other (please specify brand and/or DIN / NHPN):								

Comments:

4. From the list below (show participant the list), please tell me all of the following **herbal/ botanical** or **animal derived** supplements that you are currently taking.

Dietary Supplement	**1st Visit** **Time Point:**		**2nd Visit** **Time Point:**		**3rd Visit** **Time Point:**		**4th Visit** **Time Point:**	
Herbal supplements:	✓	Dose / Frequency / Place where they bought them	✓	Dose / Frequency / Place where they bought them	✓	Dose / Frequency / Place where they bought them	✓	Dose / Frequency / Place where they bought them
Aloe Vera								
Astragalus								
Bilberry								
Black Cohosh								
Black Elderberry								
Blue Cohosh								
Brewer's Yeast								
Cascara Sagrada								
Castor Oil								
Cat's Claw								
Cayenne								
Chamomile								
Chinese Herbs								
Cinammon								
Cod Liver Oil								
Cold FX								
Cranberry								
Dong Kuai (Tangkwei)								
Dried Cherry								
Echinacea								
Ephedra								
Evening Primrose Oil								

Fish Oil (fatty acids)								
Feverfew								
Flaxseed Oil								
Garlic								
Ginger								
Ginko Biloba								
Ginseng (American or Asian)								
Goldenseal								
Grapeseed Extract								
Horsetail								
Kava, Kava								
Milk Thistle								
Peppermint								
Pregnancy Tea								
Pumpkin Seeds								
Raspberry Leaf								
Saw Palmetto								
Siberian Ginseng								
Slippery Elm								
St. John's Wort								
Valerian								
Homeopathic Remedies (please specify):								
Other (please specify brand and/or DIN / NHPN):								

Comments:

Finally, may I see the containers, bottles, labels or bags for **all** the prescription and nonprescription vitamins, minerals, and dietary supplements that you are currently using?

In case you do not have it with you at the moment, would you bring **all** of them to your next appointment?

SECTION II. WATER INTAKE.

The following questions are about your everyday water intake.

1. On a daily basis, which types of water do you usually drink? And what is the average intake (glasses/day) from the mentioned source(s)? Please check all that apply. 1 glass = 250 ml. / 8 ounces.

Sources	**1st visit** **Time Point:**		**2nd visit** **Time Point:**		**3rd visit** **Time Point:**		**4th visit** **Time Point:**	
	✓	**Intake**	✓	**Intake**	✓	**Intake**	✓	**Intake**
Bottled water								
Sparkling water								
Tap water								
Tap water (with filter)								
Other(s) please specify:								

References

1. Natural Health Products System. Standard Terminology Guide. Health Canada. November 2008. Version 1.0.
2. APrON Supplement Intake Questionnaire was adapted from Health Canada and Statistics Canada - **"**Canadian Community Health Survey - Nutrition (CCHS)" 2004, NHANES - "Dietary Supplements and Prescription Medication Survey" 2005-2006, and "The Tomorrow Project" 2006 questionnaires.

Appendix 8: Nutrient Assessment

Dietary Reference Intakes

To determine whether nutrient intake meets required (i.e. adequate) levels for health and function, individual and group measures can be compared to the recommended requirements for specific nutrients, known as the Dietary Reference Intakes (DRI). The DRIs are used to assess the probability of adequacy for individuals and the prevalence of inadequacy for a population (381).

Definitions of the DRI

The DRIs were developed by the Food and Nutrition Board at the Institute of Medicine of The National Academies (formerly National Academy of Sciences). The DRI is the general term for a set of reference values used to assess nutrient intakes of healthy people. The DRI provides a distribution of requirements (not just a single number) that is described by a median and standard deviation (the estimated average), and the point on the distribution where daily recommended amount (the recommended daily allowance) would be located (381). Table 16 gives the four DRIs, their definitions, and the nutrients that have been classified with one or more of the DRIs.

Table 16: Definition of DRI terms and nutrients with respective published DRIs (381)

DRI	Definition	Nutrients
Recommended Dietary Allowance (RDA)	The average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals in a particular life stage and gender group	Vitamins B1, B2, B3, B6, B12 and folate; vitamin A, C & E; selenum, zinc, iodine iron, copper, phosporus, magnesium, molybdenum
Estimated Average Requirement (EAR)	The average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and	Vitamins B1, B2, B3, B6, B12 and folate; vitamin A, C & E; selenum, zinc, iodine iron, copper, phosphorus, magnesium,

DRI	Definition	Nutrients
	gender group	molybdenum
Adequate Intake (AI)	The recommended average daily nutrient intake level assumed to be adequate -- used when an RDA cannot be established	Calcium, vitamin D, flouride, vitamin B5, biotin, choline, vitamin K, chromium, manganese
Tolerable Upper Intake Level (UL)	The maximum daily nutrient intake level unlikely to cause adverse health effects to almost all individuals in the general population	Calcium, phosphorus, magnesium, vitamin D, flouride, B3, B6, folate, choline, vitamin A, C, E, selenium, boron, copper, iodine, iron, manganese, molybdenum, nickel, zinc

To illustrate the use of the DRIs, a usual intake above the RDA is considered "probably adequate", since it meets the requirement of 97-98% of individuals and only has a 2-3% chance of being inadequate. On the other hand, an intake at or above the EAR does not convey a "probably adequate" intake, since the EAR is an estimate of intake meeting the requirement of only about half of individuals. Thus, the EAR alone cannot be used as a measure of adequacy. If the EAR or RDA is not available for a particular nutrient, then the AI must be used. The AI represents intake, rather than requirement, thus it is likely to exceed the actual (but unknown) requirement (382). The AI is derived when there is not enough data to establish an EAR or RDA for a given nutrient. AI is based on experimentally derived intake levels in healthy individuals that meet criteria such as normal growth or normal levels in plasma. Levels above the AI are considered adequate, but levels below AI must be interpreted with caution, i.e. "no quantitative estimate can be provided of the likelihood of inadequacy" (382). Tables 17a and 17b give the values for the DRIs in pregnant women.

Table 17a: Micronutrients and their DRIs (EAR, RDA, AI, UL) in pregnant women

Nutrient (citation)	EAR	RDA	AI	UL
Vitamin B1 (382)	1.2 mg	1.4 mg	--	ND
Vitamin B3 (382)	14 mg	18	--	35 mg
Vitamin B6 (382)	1.6 mg	1.9 mg	--	100 mg
Vitamin B12 (382)	2.2 µg	2.6 µg	--	ND
Folate (382)	520 µg	600 µg	--	1000 µg
Vitamin C (382)	70 mg	85 mg	--	2,000 mg
Vitamin D (3)		15 µg (600 IU)	5 µg	1,000 µg
Vitamin E (382)	12 mg	15 mg (22.4 IU)	--	1,000 mg
Iodine (382)	160 µg	220 µg	--	1,100 µg
Iron (382)	22 mg	27 mg	--	45 mg
Magnesium (382)	300 mg	360 mg	--	350 mg
Selenium (382)	49 µg	60 µg	--	400 µg
Zinc (382)	9.5 mg	11 mg	--	40 mg
α-Linolenic acid (383)	--	--	13 g	ND

ND = not determined

Table 17b: Non-Institute of Medicine DRI-derived recommendation of DHA for pregnant women

Nutrient (citation)	Recommended daily amounts
DHA (384)	200-300 mg (source: WAPR, ENA, CHF)

WAPM = World Association of Perinatal Medicine, ENA = Early Nutrition Academy, CHF = Child Health Foundation

When applying the DRI values, the terms *adequate* and *sufficient* intake have been used interchangeably in the literature, and represent levels at or above requirement. The consequence of *inadequate* or *insufficient* intake (below requirement but evidence of *some* intake) for a particular nutrient is generally unknown, although possible associations with certain chronic diseases have been implicated. *Deficient* intake often refers to a lack of intake of a nutrient where disease can result. The Institute of Medicine in the U.S. has the following definition of terms in assessing nutrient intakes (382):

- *Distribution of requirements* – "the distribution that reflects the individual-to-individual variability in requirements. Variability exists because not all individuals in a group have the same requirements for a nutrient, even if the individuals belong to the same life stage and gender group";
- *Probability of inadequacy* – "the outcome of a calculation that compares an individual's usual intake (long-term) to the distribution of requirements for people of the same life stage and gender group; used to determine the probability that the individual's intake does not meet his/her requirement";
- *Probability of adequacy* – "100 percent minus the probability of inadequacy";
- *Prevalence of inadequacy* – "the percentage of a group with intakes that fall below requirement".

There are two general methods to assess the prevalence of intake inadequacy for groups: 1) the probability approach, and 2) the EAR cut-point method. The probability approach is "a statistical method that determines the probability of inadequacy of the usual intake level for each person in the group and then averaging these individual probabilities across the group to obtain an estimate of the group's prevalence of inadequacy". The two assumptions associated with this

approach are that intake and requirement are independent (i.e. no correlation between the two variables), and that the distribution for the nutrient requirement is known. The EAR cut-point method is derived from the probability approach and involves counting the number of individuals in the group whose usual intake is below the EAR, and this proportion will reflect those that do not meet their requirement for a given nutrient (385). To use this method, these assumptions must be met: 1) intakes and requirements are not correlated; 2) distribution of requirements is symmetrical; 3) the standard deviation of intake distribution is greater than the standard deviation of requirement distribution (which is likely true in free living individuals but not for groups with similar diets such as prison inmates). If the three assumptions cannot be met, then the probability approach must be used (382).

The following guidelines are provided by the Institute of Medicine in the U.S. for using the DRIs to assess group nutrient intakes (382):

- For nutrients with an EAR, the EAR can be used to estimate the prevalence of inadequate intakes using the probability approach or the EAR cut-point method;
- The RDA is inappropriate for assessing nutrient intakes of groups because the RDA is the intake level that exceeds the requirements of a large proportion of individuals in a group. Determining the proportion of individuals with intakes below the RDA will overestimate the true prevalence of inadequacy;
- For nutrients without an EAR, use the AI; groups with mean or median intakes at or above the AI can be assumed to have low prevalence of inadequate intakes;
- For nutrients with a UL, it can be used to estimate the proportion of a group at potential risk of adverse effects from excessive nutrient intake.

Table 18: Proportion of women from the APrON cohort meeting or exceeding the UL for micronutrients from supplements

Micronutrient from supplements	Percent at/above UL (n = 601)
Vitamin B1 (mg)	ND
Vitamin B3 (mg)	7.3%
Vitamin B6 (mg)	1.8%
Folic acid B9 (µg)	85.9%
Vitamin B12 (µg)	ND
Vitamin C (mg)	0.5%
Vitamin D (IU)	18.5%
Vitamin E (mg)	0.3%
Iodine (µg)	0.3%
Iron (mg)	17.6%
Magnesium	0.5%
Selenium (µg)	0.3%
Zinc (mg)	1.7%
Omega-3 (mg)	ND

Types of Dietary Intake Measurements

For individual-based studies, researchers may use one or more methods for assessing dietary intake. These methods provide a systematic estimation of food consumption, taking into account frequency, portion size, ingredients, combinations, and cooking form, which could all affect exposure to certain dietary components (386). There is no single ideal method, as each method has its strengths and weaknesses as presented below (387).

The *Food Frequency Questionnaire* (FFQ) assesses an individual's consumption over a certain period of time (e.g. past month or year). Semiquantitative FFQ includes portion sizes to determine quantities of food eaten and/or nutrient intake. FFQs can be brief, consisting of a short list of foods or specific nutrients of interest (e.g. 10 – 20 items), or they can be comprehensive, with a larger list of foods or nutrients (e.g. 100 – 150 items) being consumed. The benefits of the FFQ are that it can estimate foods usually eaten over a given period, rank high verses low intakes of specific foods or nutrients, be self administered, and be relatively inexpensive compared to other methods. The drawbacks of the FFQ are that it requires participant recall, the recall period is imprecise, quantification may be inaccurate due to poor estimation of portion sizes, the food lists may miss important foods or nutrients, and the FFQ is usually close-ended, thus, not capturing foods that are common to the participant's diet.

The *food (diet) records* ask participants to record their food intake over a number of days, typically 3, which is the preferred standard for measuring food intake. The quantities of foods consumed are measured using standard measuring devices (e.g. cups, tablespoons) or food models (e.g. pictures). Food intakes are recorded at the time of consumption (or very close to it), and participants are trained to record the details of their meals. The benefits of this method are a higher rate of accuracy due to the immediacy of data collection (thus, omission of foods is

minimal), the use of standard measures to accurately capture quantity of foods consumed, and its open-ended nature that allows for the identification of a variety of foods consumed that are specific to individual patterns and habits. One drawback of this method is a high respondent burden that requires participants to be cooperative, motivated, and have high literacy skills. As well, reliability may diminish over time due to respondent fatigue, and respondents may not record their food intake immediately, thus leading to an increase in error rates over time.

The *24 hour food (dietary) recall* asks participants to recall all of the food and beverages they had consumed over the preceding day (24 hours). An interview format is often used, which may be face to face or over the phone. Well trained interviewers are required who are familiar with the eating habits of the participants, so that detailed, accurate, and complete answers can be extracted. The benefits of the 24 hour recall are an increase in reliability of the data due to its open-ended nature, an interview process that allows for clarification and detailed data collection, minimal literacy requirement, and a relatively low respondent burden. Drawbacks to this method are that it has potential for recall bias, the portion sizes may be inaccurate, and it requires trained interviewers. As well, because of day to day variations, a single 24-hour recall is not adequate for measurement of an individual's usual intake. To increase accuracy, multiple recalls are often used. However, a single 24- hour recall from each individual can be used to estimate the average nutrient intakes of groups rather than of specific individuals (388).

The *food (diet) history* is a combination method that starts with a 24-hour recall interview, is followed by completing a FFQ, and then finishes by collecting a 3 day food record. Variations of this method are used. For example, some studies have abandoned the 3 day food record. Because of the multiple forms of data collection, this method is time and resource

intense and exerts a high respondent burden. The benefits and drawbacks of this method correspond to those of the individual methods used, as previously mentioned.