PREGNANCY, NEONATAL AND DISEASE OUTCOMES IN WOMEN WITH RELAPSING REMITTING MULTIPLE SCLEROSIS

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

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Leslie Dan Faculty of Pharmacy

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2018

ABSTRACT

Objective

To compare pregnancy, neonatal and disease outcomes in women with Relapsing Remitting Multiple Sclerosis (RRMS) exposed to different therapeutic agents—natalizumab (Tysabri®) and Vitamin D.

Methods

Data from German and Canadian Multiple Sclerosis (MS) pregnancy registries was compared to evaluate neonatal and pregnancy outcomes in natalizumab exposed and unexposed groups. A healthy control group of pregnant women unexposed to teratogens was also utilized; primary outcome of interest was birth defects and miscarriage rates. For the Vitamin D study we first compared postpartum disease outcomes using Kaplan Meier and Cox Regression in a German (GER) cohort not supplementing with Vitamin D to a Canadian (CAN) cohort with Vitamin D
supplementation in pregnancy; primary outcome of interest was time to disease resurgence in the postpartum period. We then evaluated neonatal outcomes in Canadians with high dose Vitamin D (>4000IU/day) in pregnancy to ones with lower intake. Primary outcome of interest was birth weight.

Results

There were no significant differences in birth defects or other adverse neonatal outcomes between the 101 natalizumab exposed, 78 disease matched, and 97 healthy controls. The rates of miscarriage were higher in both MS groups in comparison to the healthy controls. In the Vitamin D study, significantly more GERs used Disease Modifying Drugs (DMDs) in pregnancy and postpartum compared to CANs. There was no difference in disease resurgence between the two cohorts. Neonatal outcomes including birth weight did not differ significantly in the 29 women that exceeded 4000IU/day in pregnancy, compared to women that took lower doses.

Conclusion

Gestational exposure to either therapy, natalizumab or Vitamin D, is not associated with adverse outcomes. Larger studies are needed to investigate the longer duration of therapy with natalizumab and higher doses of Vitamin D >4000IU/day in pregnancy.
Acknowledgments

“Those things that hurt, instruct.” —Benjamin Franklin

In January 2013, I formally began my PhD journey, after a year of speaking to many incredibly brave women choosing to become mothers in spite of their MS—un-submitting to the ambivalence surrounding their disease. They sparked in me inspiration, curiosity and a burning ambition that made me pursue this topic. I happily committed 12-15 hour days for the next two years interviewing patients and published my first paper; assured by my progress pace, that I will complete this academic milestone in three years if not less; and how arrogant I was for believing this.

March 2015 brought me to a complete halt and for the Nth time I re-learned the naivety and triviality of “planning” and “hard work” in this tsunami of life. Yet, as someone accustomed to adversaries, I was fortunate enough to learn a new lesson on the value and magnificence of kindness from those unrelated to you.

Over the last two years riding what felt like a sinking ship at SickKids, my committee members became my life jackets. Dr. Ann Yeh, Dr. Richard Gladstone and Dr. Manny Papadimitropoulos have gone above and beyond their role as advisors to propel me forward. Their unwavering support and encouragement, contribution of expertise in critically evaluating my work and strategizing, taking the time to meet with me in spite of their incredibly hectic schedules, are the very reasons how I came from halt to crawl to the finish line. Words fail me in thanking you and I am forever indebted to you and all that you have done.

Dr. Shinya Ito, stepped in at the peak of chaos to become my supervisor, adding more weight to the already overbearing load put on him, and provided the structure and support I needed to continue on. I am eternally grateful for your expertise and guidance.

My Motherisk family, the counsellors and students have seen me through nine years of personal and academic growth and been by my side every step
of the way. Thank you for the laughter, the unforgettable memories, and the lifelong friendships. Special thanks to Pina Bozzo for the kind of bond, friendship and love that is irreplaceable, and to Dr. Irena Nulman for being a force greater than life, inspiring and motivating me with her infinite wisdom and humanity.

My family, particularly my husband Miguel, have patiently and optimistically cheered me on and endlessly believed in me when I was swathed in pessimism; and four years and 8 months later, I’m fortunate enough to end this journey with them by my side.

Lastly, I want to thank all the incredible women that spent hours talking to me sharing their experience, with no incentive but to broaden our understanding of this disease and help other women with MS. Had it not been for these long detailed and very personal conversations, I would not have grasped the depth and impact of this disease and I am grateful for their trust and enthusiasm in my work and I. I look forward to the bright future ahead of us, and confident that the cure to many of these diseases will be discovered; I for one, vow to play my part in finding these answers. Thank you for teaching me the skills to do so.

I dedicate this thesis and all the future work I partake in, to these women and the spirit of motherhood.
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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapsing Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>CIS</td>
<td>Clinical Isolated Syndrome</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>SPMS</td>
<td>Secondary Progressive MS</td>
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<tr>
<td>BF</td>
<td>Breastfeeding</td>
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<tr>
<td>EBF</td>
<td>Exclusive Breastfeeding</td>
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<tr>
<td>BW</td>
<td>Birth weight</td>
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<td>BW%</td>
<td>Birth weight Percentiles</td>
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<tr>
<td>LBW</td>
<td>Low Birth Weight</td>
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<tr>
<td>SGA</td>
<td>Small for Gestational Age</td>
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<tr>
<td>IUGR</td>
<td>Intrauterine Growth Restriction</td>
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<tr>
<td>HC</td>
<td>Head Circumference</td>
</tr>
<tr>
<td>SA</td>
<td>Spontaneous Abortions/ Miscarriage</td>
</tr>
<tr>
<td>TA</td>
<td>Therapeutic Abortions/Terminations</td>
</tr>
<tr>
<td>C/S</td>
<td>Cesarean Section</td>
</tr>
<tr>
<td>DMD</td>
<td>Disease Modifying Drugs/Therapies</td>
</tr>
<tr>
<td>Calcidiol</td>
<td>25-Hydroxyvitamin D / 25(OH)D</td>
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<tr>
<td>25(OH)D</td>
<td>Calcidiol</td>
</tr>
<tr>
<td>1,25(OH)_{2}D</td>
<td>1,25 dihydroxyvitamin D</td>
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<tr>
<td>Calcitriol</td>
<td>1,25(OH)_{2}D</td>
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<td>CAN Cohort</td>
<td>Canadian Cohort</td>
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<tr>
<td>GER Cohort</td>
<td>German Cohort</td>
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<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>FS</td>
<td>Functional Systems</td>
</tr>
<tr>
<td>EBV</td>
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CHAPTER I

BACKGROUND

Multiple Sclerosis

*What is Multiple Sclerosis?*

Multiple Sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS) and the most common cause of neurological disability in young adults in Western countries. In MS, the myelin sheaths of the central nervous system (CNS) are targeted by immune cells, promoting inflammation and scarring that lead to loss or compromised signal transmission in nerve fibers. Neurological deficiencies vary significantly between individuals and depend on site of damage and size of lesions. Symptoms may present as sensory, cognitive, bladder/bowel, balance and mobility dysfunction. Depression, fatigue and pain are also commonly experienced. Studies on the natural history of MS course suggest a more aggressive disease course in men, patients with higher relapse frequency and shorter interval between relapses in the first two years of onset, incomplete recovery from the first relapse, and higher age at disease onset. While poly-symptomatic presentation has not been predictive of worse disease, higher lesion number and volume at onset, and specifically brainstem lesions were associated with worse disease outcomes. The severity of impairments can range from minimal to extreme with progressive worsening over time. From disease onset onward, frequency of visits to the emergency room, healthcare providers (physicians, physical, occupations and speech therapists) and hospitalization is substantially higher in MS patients compared to healthy individuals. The economic burden of the disease is one of the highest among all chronic disease, with an estimated lifetime cost of $4.1 million per patient in the United States. In Canada the total direct
costs (hospitalization, physician care, and drug expenditure) associated with MS in 2000-2001 was an estimated 139.2 million.\textsuperscript{6} Thus in addition to significant disability endured by MS patients, the early age of onset, chronicity and progressive nature of this disease, translate into significant societal and familial burden.

\textit{Multiple Sclerosis Prognosis}

MS is a chronic, unpredictable and progressive disease. There is no single clinical feature nor diagnostic test to quickly detect MS. The diagnosis is based on clinical parameters with MRI imaging and other para-clinical tests used for ascertainment. The 2010 McDonald criteria for MS diagnosis requires a combination of clinical attack(s) along with MRI evidence of dissemination in space (presence of at least one T2 lesion in two of the four CNS areas—periventricular, juxtacortical, infratentorial or spinal cord) and MRI evidence of dissemination in time (new T2 or gadolinium enhancing lesions on subsequent MRIs).\textsuperscript{7} Differentiating between MS and other causes of brain and spine MRI abnormalities is a key first step in the assessment of a patient presenting with complaints suggestive of MS. Conventionally, upon the first clinical episode, characterized by inflammatory demyelination indicative of MS, a patient may be diagnosed with Clinically Isolated Syndrome (CIS). A second episode (either an exacerbation or MRI activity) may lead to diagnosis of definite MS.\textsuperscript{8} In October 2017, revisions were made to the 2010 McDonald’s criteria to allow for: 1) earlier diagnosis of MS, 2) distinction of MS from other demyelinating diseases that have overlapping clinical and MRI features such as Neuromyelitis Optica Spectrum Disorder, 3) incorporation of cerebrospinal fluid oligoclonal bands and other para-clinical test findings as key diagnostic tools rather than secondary and confirmatory evidence and 4) less stringent criteria for dissemination in time and space.\textsuperscript{9} MS may further be categorized into several distinct categories. In 2013 the US National MS Society (NMSS) advisory committee, revised definitions
of MS subcategories and included four distinct clinical courses: 1) Relapsing Remitting MS (RRMS), 2) Secondary Progressive MS (SPMS), 3) Primary Progressive MS (PPMS) & 4) Progressive Relapsing MS (PRMS). Relapses are the most universal feature of MS. They are defined as acute or subacute episodes of new or worsening neurological dysfunction lasting greater than 24 hours, followed by full or partial recovery in the absence of fever and/or infection. After an initial neurologic event, most patients will be defined as having RRMS. 80% of these individuals will eventually enter the secondary progressive (SPMS) phase, with 50% reaching this phase in the first 10-15 years. SPMS is defined as a progressive stage marked by gradual worsening of symptoms without any recovery and may or may not entail acute relapses. The transition from RRMS to SPMS is unpredictable and varies considerably in duration between individuals, but appears to be gradual. To date, a specific clinical transition point has not been identified and the diagnosis of SPMS is made retrospectively according to patient history. Primary Progressive MS (PPMS) is a distinct and non or less inflammatory pathologic form of MS. It differs from SPMS in that it is not preceded by a relapsing/remitting phase. 15% of patients diagnosed with MS experience this disease course, which typically has a later onset than RRMS and is marked by progressive accumulation of disability from onset of MS with few if any plateaus, remissions, and minor improvements. In contrast, Progressive-Relapsing MS (PRMS) displays the same progressive accumulation of disability, but is accompanied with clear acute clinical relapses with or without full recovery. Only 5% of patients fit this subtype. The progressive stage of MS is marked by failure to re-myelinate, continued excitotoxicity and apoptosis leading to neuro-axonal damage and brain tissue atrophy. The clinical consequence of this is irreversible disability accumulation. Disease progression follows a varied course in individuals. Requiring assistance to walk, being bedridden, and death are some major disability milestones that patients confront.
The Expanded Disability Status Scale (EDSS) is the most common disability assessment tool used in MS. The scale was developed by Dr. John F. Kurtzke, and provides a rating for neurological impairment in MS, based on 8 functional systems (FS)—pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and other.\textsuperscript{11} EDSS scores range from 0-10 with zero indicating a normal neurological exam and 10 indicating death due to MS. Table 1 in the Appendix defines the degree of disability attributed to each score. Typical endpoints of interest in epidemiological studies of MS progression, is time to reach EDSS Scores 3.0 (moderate disability in one FS), 4.0 (fully ambulatory despite severe disability, able to walk 500m), 6.0 (requiring unilateral assistance to walk 100m without resting), 8.0 (restricted to bed/chair), or conversion to SPMS.\textsuperscript{12}

In a 2016 study on the clinical course of MS in a Swedish cohort of 12,703 patients, the mean age at disease onset was 33±11 years and the median time to reach EDSS 3.0, 4.0 and 6.0 and SPMS were 19.8, 26.2 and 30.4, 23 years respectively.\textsuperscript{12} The median ages for these outcomes were 55, 60, 64 and 57 years of age. The median age at death from all-cause mortality was 80.5 years. Men typically had a 30% higher risk for conversion to SPMS and did so at an earlier time. Men also had a 45% higher risk of mortality due to MS compared to women. Hence while many patients may live until the eighth decade and beyond, MS confers a risk of earlier mortality (mortality risk was 2.02 times greater in MS patients compared to age and sex matched individuals in the general Swedish population). A significant reduction in life quality also occurs due to moderate to severe disability spanning 2-3 decades of these individuals’ lives.\textsuperscript{12}

\textit{Incidence and Prevalence}

2.3 million people worldwide live with MS.\textsuperscript{13} The prevalence is highest among those with northern European ancestry, and those living in temperate regions: Canada, UK, Finland, Germany, Denmark, United States, Norway.
and Sweden, have especially high rates. Recent studies in the United States suggest that incidence in African decent individuals have increased and is now similar to Northern European descent individuals living in United States. Canada displays the highest incidence and prevalence rates of MS in the world, 13.5 & 291 per 100,000 respectively. Women are three time more likely to be diagnosed with MS with some studies suggesting an increasing gap. Typical onset age of MS is between 20-30 years, and diagnosis usually occurs before age 40 during reproductive years.

**MS Etiology**

The cause of MS is unknown, but accumulating evidence suggest it results from an interplay between genetic and environmental factors. The genetic contribution is observed in the prevalence of the disease within families. The risk increases from 0.1-0.2% in the general population to 1.2-2.3% when a first degree relative has MS. The Major Histocompatibility complex (MHC) is a gene that encodes MHC Class I & Class II molecules, responsible for presenting foreign antigen to T-cells, triggering immune response against antigens. This gene is highly polymorphic, with several hundred allelic variants, purposed to expand the antigen recognition diversity of MHC molecules many fold. Certain genetic variants are now recognized as “risk genes” in MS, and over 200 have been identified. All 200 are single nucleotide polymorphisms in genes responsible for coding innate and adaptive immunity, hence a wide range of immune cell types may play a role in MS pathogenesis. The first and largest studied variant is an allelic variation in the HLA-DRB1 chains from MHC class II; the HLA-DRB1*15:01. This risk gene has been shown to increase MS risk by up to 3 fold (other risk genes contribute a 1.2 fold increase only. The variant 15:01 of the HLA-DRB1 is expressed in antigen presenting cells, and plays a direct role in regulating CD4 T-cells response, by
presenting Epstein Bar Virus (EBV) antigen. Unlike other DRB1 alleles, this variant can also bind myelin protein, driving the attack on self-antigen as seen in MS. The discovery of the risk genes highlight the complexity of dysregulation in multiple immune systems, but only partially explain susceptibility to MS. Genome wide association studies suggest that risk genes only explain 50% of the variation in developing MS. The most concrete evidence for the limitation of genetic play comes from identical twin studies where disease risk is 25% (and not 100%) when one twin is affected by MS.

It is thought that the interplay between genetics and environmental factors lead to defects in peripheral tolerance of CNS antigens. Impairments in regulatory T cells and suppression of autoreactive effector cells lead to infiltration of the CNS through a compromised blood brain barrier, causing perivascular lesions flooding with macrophages and T-lymphocytes. These infiltrates then interact with CNS based immune cells, such as astrocytes and microglia promoting demyelination. These lesions, if active, are thought to promote relapses or manifest as radiological activity evident on MRI imaging as enhancing and non-enhancing lesions. Later stage MS is marked by more diffused inflammation and prominent neurodegeneration which appear to be mechanistically different than that observed in early stage MS.

Hence, environmental factors play an indisputable role in MS manifestation in genetically susceptible individuals. Some of the most frequently cited non-genetic risk factors include: living in higher latitudes, female gender, Caucasian ethnicity, migration to high MS prevalence areas prior to age 15, high hygiene in early childhood, Vitamin D status, obesity, infection with EBV and cigarette smoking. Derivatives of Vitamin D status (dietary intake, sunlight exposure and supplementation) have been the most notorious environmental risk factor in MS. As one of the main focuses of this thesis, particular attention is given to the role of Vitamin D in the following sections.
Vitamin D and MS

*What is Vitamin D?*

By definition, vitamins are organic compounds involved in specific chemical reactions in the body, and over the course of evolution, humans have lost the ability to synthesize them endogenously. Due to their abundance in food sources, this lack of synthesis can be compensated through diet. Truly essential compounds required by every living cell however, can still be synthesized endogenously even when food sources are abundant; cholesterol is one such example. Likewise, Vitamin D is considered an essential compound, acting as a transcription factor controlling the expression of thousands of nuclear genes throughout the body, and its deficiency is associated with many serious medical conditions, including colon cancer, diabetes mellitus, osteoporosis, obesity. The body is able to produce its own Vitamin D, and food sources are not abundant. Hence classifying this simply as a vitamin may not be appropriate. There are two physiologically active forms of Vitamin D, ergocalciferal (Vitamin D$_2$) and cholecalciferol (Vitamin D$_3$). Vitamin D$_2$ is synthesized by plants and some mushrooms, while D$_3$ is synthesized in humans by UVB irradiation of the skin and is also available from animal sources. Ultraviolet B irradiation is the most common source of Vitamin D in the world, and its intensity & duration is positively correlated to latitude. Very few food sources provide enough Vitamin D$_3$ and do so only when consumed regularly—fatty fish and reindeer meats are two examples of animal sources of D$_3$. Through UVB irradiation of the skin’s 7-dehydro-cholesterol, pre-Vitamin D$_3$ is a formed and through a secondary reaction and the restructuring of the double bonds Vitamin D$_3$ is produced. Vitamin D$_3$ and Vitamin D$_2$ undergo similar metabolism to yield calcidiol and calcitriol, through two hydroxylation reactions. In the first reaction, primarily occurring in the liver
via the 25-hydroxylase enzyme, calcidiol [25(OH)D$_3$] is formed; this is the universal biomarker of Vitamin D status, which can be tested in blood samples.$^{16}$ Vitamin D$_3$ is known to be three times more potent than Vitamin D$_2$ in raising and maintaining serum calcidiol status and has a higher affinity for VDBP in plasma.$^{21}$ A secondary hydroxylation reaction via the 1-$\alpha$-hydroxylase enzyme in the kidneys yield the active metabolite: calcitriol [1,25-(OH)$_2$D$_3$]. Many tissues in the body (skin, intestine, bones, parathyroid gland, immune cells, pancreas and fetal tissue) are able to produce calcitriol using the same enzyme.$^{21}$

Vitamin D plays an important role in calcium homeostasis. Through a negative feedback mechanism, calcitriol is able to suppress parathyroid hormone (PTH). PTH is involved in raising blood calcium levels through bone resorption, and when calcidiol levels are elevated, blood calcium levels can rise through other mechanisms that are not dependent on bone resorption.$^{21}$ This is achieved through the action of calcitriol, which leads to greater intestinal absorption of calcium and phosphorous, reabsorption of urinary calcium, scavenging calcium at the expense of phosphorous to maintain calcium status throughout the body.$^{22}$ At 80 nmol/L, PTH is maximally suppressed, leading to maximal calcium absorption, which will prevent premature bone loss and fractures.$^{21}$

Maintaining bone integrity is a well-known classical function of Vitamin D. It is an established fact that immune modulation, differentiation, proliferation and immune response is also under the direct influence of Vitamin D.$^{16}$ Vitamin D Receptors (VDR) are found on the surface of cells throughout the body including activated T Cells, B Cells and macrophages.$^{16}$ Once Vitamin D binds to the VDR the complex is internalized and forms a heterodimer with the retinoid X receptor.$^{16}$

The heterodimer is translocated to the nucleus and binds to the Vitamin D Response Element (VDRE).$^{16}$ The VDRE is located on the promoter regions of many nuclear genes, including a region adjacent to the HLA-
The immunomodulatory effects of Vitamin D in MS are under investigation, but evidence suggests that the protective role in MS may partially be attributed to increased IL-17 and the induction of regulatory T-cells. Individual calcidiol levels are influenced by many factors including: melanin levels, latitude, sunscreen use, season, air pollution, dress code, age, BMI, diet, race, and genetic makeup. These factors influence skin production of Vitamin D₃ and/or its absorption and metabolism. Melanin, for example, acts as a filter, reducing Vitamin D photosynthesis. Hence, individuals with darker skin in a community typically have lower calcidiol levels than fair skinned individuals in the same community. Fitzpatrick skin phototype, classified according to the amount of melanin pigment, ranging from I(fair)-VI(dark), determines the level of sunburn a person may endure with high exposure to sunlight. Typically phototypes I & II have significantly higher calcidiol synthesis than those with phototype VI.

**Vitamin D Deficiency: A Risk Factor for MS Onset**

A latitudinal gradient has been noted in many studies, with increasing prevalence and incidence of MS in countries furthest from the equator. One reason for this may be that the angle of UVB irradiation striking the earth becomes more oblique, and the duration and intensity of sun exposure is reduced. This makes UVB irradiation inadequate for most months of the year for sufficient Vitamin D synthesis. In recent years however the latitudinal gradient has weakened in the northern hemisphere and nearly disappeared within the United States. A more equal geographical spread of the disease is now seen. Immigration and sun avoidance may partially explain the increasing incidence of MS in sunnier climates.

The association between Vitamin D and MS risk is supported by evidence from high-risk countries such as Norway, where consumption of boiled or fried fish 3 or more times a week, a rich source of Vitamin D, in the
coastal areas, appears to confer protection. A 44% risk reduction was observed in the coastal population compared to those living inland in agricultural communities.

More compelling evidence for the role of Vitamin D in MS pathogenesis came from two large nested case-control studies, where serum calcidiol levels prior to MS onset were collected and compared to a healthy control population (matched for sex, age, race and timing of sample collection). The first study was conducted in US military personnel, 257 with MS and 514 controls. In the white non-Hispanic population, a 62% reduction in MS risk was observed in persons with serum D levels >99 nmol/L compared to those with less than 63 nmol/L. The second study was a Swedish study where pre MS onset samples were drawn from 192 individuals and compared to 384 controls, a 61% reduction in risk was observed in individuals with serum D levels ≥75nmol/L compared to <75nmol/L. Genome wide studies have identified four genes as the most significant predictors of circulating calcidiol—CYP2R1, DHCR7, GC, CPY24A1, all involved in Vitamin D metabolism. A higher number of alleles in these genes is associated with lower serum calcidiol levels, strengthening the relationship between Vitamin D and MS risk.

MS risk in obese patients offers further evidence for the role of Vitamin D in MS pathogenesis. Several case control studies have shown that obesity, particularly at a younger age, is associated with an increased MS risk of 60-300%. In a longitudinal study in over 300,000 individuals in Copenhagen with birthdates in the 1930s and with school health records documenting BMI every year between ages 7-13, 774 were diagnosed with MS later in life. Amongst the cases, individuals whose BMI was ≥95th % during that time, had a 60-95% increased risk for MS. In another US based study, obesity at age 20 was associated with a 2.2 fold and 2.1 fold increased in MS risk in 1571 women and men respectively. In a Southern California study, MS risk was 2 fold higher in women who were obese at ages 10-20 or
20-30, and within the same group, girls with BMI ≥35kg/m² had a threefold higher risk for onset of pediatric MS and CIS.\textsuperscript{14}

While immunomodulatory properties of adipose tissue and other complications associated with obesity may contribute to the observed MS risk in obese cohorts, the significantly lower serum calcidiol levels in these individuals support an interaction between obesity, Vitamin D and MS pathogenesis.\textsuperscript{14} As one of the most easily modifiable risk factors, it has been suggested that up to 44\% of MS cases may be avoided with Vitamin D supplementation.\textsuperscript{14} Some evidence suggest that maternal calcidiol status in pregnancy may be one maternal factor that plays a role in MS pathogenesis in offspring, and that correcting deficiency may need to be addressed much sooner than childhood and adolescent years.\textsuperscript{28}

**Vitamin D and Clinical Outcomes in MS**

A growing body of research has focused on the role of Vitamin D in disease progression and outcomes in MS. Several observational studies have demonstrated an association between higher serum calcidiol levels and more favorable disease course.\textsuperscript{23,29-32} A secondary analysis of data from a randomized control trial (RCT) with Interferon Beta-1b, reported that serum calcidiol was inversely correlated to total number of new active lesions from baseline.\textsuperscript{33} In this study a 50 nmol/L increase in serum calcidiol showed a 31\% reduction in risk of newly acquired active lesions (sum of new T2 and new gadolinium enhancing lesions), and the strongest effect was observed in patients with serum calcidiol levels above 100nmol/L with a 47\% reduction in risk for newly acquired lesions compared to patients with serum levels 50-79nmol/L.\textsuperscript{33} Other studies showed a lower conversion to definite MS in patients with CIS during the 4-7 year follow up period.\textsuperscript{23,32,34} Other studies have reported an association between higher serum calcidiol levels and lower number of gadolinium enhancing lesions and fewer relapses.\textsuperscript{30,31,35} Correcting insufficient levels early in disease course appears to confer the
most protection, and while optimal serum levels of calcidiol have yet to be elucidated, serum levels ≥100nmol/L appear to reduce risk of acquiring new active lesions and decrease T-cell proliferative response.\textsuperscript{33,36} 100 nmol/l can be achieved with 1000-4000IU/day of Vitamin D supplementation without risk of hypercalcemia.\textsuperscript{14,33} A 2013 Meta-analysis of 129 MS patients from 5 RCTs, examined the effect of supplementation with any formulation or dose of Vitamin D on relapse risk.\textsuperscript{37} The treated groups (N=129) in these trials were on a higher dose of either Vitamin D\textsubscript{2} (6000Iu/day), D\textsubscript{3} (dose: 2800-40,000IU/day) or calcitriol (0.25-0.5µg/day) administered over different time durations (6-22 months). The controls consisted of MS patients (N=125) either on a low dose of Vitamin D [dose: 1000IU/day of D\textsubscript{2} (N=12) or 4000 IU/day D\textsubscript{3} (N=23)] or placebo (N=90). No impact on relapse risk was found in this meta-analysis 0.98 (95% CI 0.44–2.17). The authors acknowledged that variability in cohorts, small sample sizes in most trials and differences in treatment type and duration, may have contributed to this finding. Baseline levels in MS patients averaged around 54-59 nmol/L with some already above 75nmol/L in these trials, likely leading to plateauing effect of any potential benefits of Vitamin D supplementation.

**MS and Pregnancy**

**MS Course in Pregnancy**

Many studies have found pregnancy to be a protective state for women with MS. The first large prospective study conducted by Confavreux et al. in 1998, called the Pregnancy in MS (PRIMS) study, followed up 269 pregnancies reported by 254 women with MS from 12 European countries from one year prior to conception, throughout pregnancy and up till 12 months postpartum.\textsuperscript{38} The annual relapse rate dropped from 0.7/year prior to pregnancy to 0.2/year in the third trimester and increased to 1.2/year in the first three months postpartum.\textsuperscript{38} Only 28% of their women experienced postpartum relapses, and the risk for relapse was highest in the first three
months postpartum, and not significantly different between 3-12 months postpartum compared to the pre-pregnancy state. Similar patterns have been reported in other epidemiological studies. A meta-analysis in 2011 reported relapse outcomes in 1221 pregnancies from 13 studies, confirming that relapse rates decrease significantly during pregnancy (from 0.44/year in the pre-pregnancy year to 0.26 during pregnancy) and increase significantly after delivery (0.76/year). The frequency and course of relapses postpartum is not predictable, although some association with relapse rate in the year prior to pregnancy, and disease duration from diagnosis has been suggested. A recent study reported that in addition to pre-conception relapses, relapse frequency during pregnancy and EDSS scores of two or more at time of conception were associated with higher risk of postpartum relapses, while DMD exposures within 2 years of conception reduced this risk.

Hormonal changes (i.e. increased estrogen) and non-hormonal mechanisms specific to the pregnancy state are thought to be responsible for relapse reduction during pregnancy. Circulating levels of estrogens, progesterone, glucocorticoids and Vitamin D, all of which have demonstrated immunomodulatory properties, undergo marked shifts during pregnancy and postpartum. While high estrogen levels have shown to be protective in experimental autoimmune encephalitis (EAE), the animal model for MS, progesterone on its own despite demonstrating re-myelinating properties and prevention of axonal loss, has not shown promise. In contrast the combination of progesterone with estrogen seems to have an additive response in conferring protection in EAE mice. The effect of Vitamin D has been demonstrated in EAE, and more interestingly, in the presence of estrogen, the protective effect of Vitamin D seems to be enhanced in animal models of MS.

Apart from pregnancy hormones, changes in maternal immune response may provide another explanation for decreased numbers of relapses
observed in pregnancies in women with MS. Decrease in pro-inflammatory gene expression and a shift from Th1 to Th2 cells occurs during MS pregnancies with a subsequent increase postpartum. A decline in Interleukin-8 (IL-8), an inflammatory chemokine secreted by macrophages, and INF-γ secreting CD4+ T cells during pregnancies in women with MS have been documented. T-regulatory (Treg) cells, are a subset of CD4+ T cells, known to suppress deleterious effects of Th cells. In doing so, Treg cells play an important role in tolerance to self-antigens (preventing autoimmune disease) and fetal tolerance during pregnancy. A progressive increase in Treg cells during pregnancy is observed, and in a small study of 13 MS pregnancies, a significant increase in Treg population in both healthy and MS pregnancies, as compared to the non-pregnant state was reported. In this study the percentage and absolute count of Treg cells were higher in early pregnancy and postpartum of MS patients compared to the healthy controls. These findings in Treg expression in human MS pregnancies have not been replicated in other studies. Small sample size, differences in timing for measuring markers, and defining Treg subpopulations may have been some of the reasons for the lack of replication.

Maternal-fetal factors may also contribute to lower relapse rates in MS. During pregnancy, changes in maternal immunity protects the embryo, a “foreign” body, developing inside the mother. This maternal tolerance of the semi-allogeneic fetus, may at least partially be attained through to the direct interaction between fetal antigen and maternal Treg cells, shaping maternal immunity and reinstalling or increasing tolerance to self-antigen in the mother. If pregnancy is considered to be a temporary treatment for MS, the question arises as to whether subsequent pregnancies lead to better outcomes in women with MS. The relationship between parity and long-term outcomes such as reaching the progressive phase of the disease or irreversible disability, i.e. an EDSS of 4 or 6, has been investigated. A delayed onset of
progression in parous women compared to women who never became pregnant has been reported. These studies are limited in their inadequate matching for disease severity, duration and age at baseline, suggesting the potential for selection bias and reverse causality. If a true effect exists, and each pregnancy does indeed confer lasting protection to women with MS, one may postulate that this may be due to persistence of Treg cells induced by fetal antigens, or pregnancy-hormone mediated mechanisms such as re-myelination, synaptic plasticity, and neuronal sparing.

Given the complexity of pregnancy dynamics, it is unlikely that one factor contributes to relapse reduction alone and hence replicating the protective effect of pregnancy in the postpartum period, or in non-pregnant MS patients, including men, has not yet been successful. However a better understanding of changes in pregnancy, immune response and fetal tolerance will provide invaluable insight on mechanisms that contribute to the remission observed in MS and some other autoimmune diseases such as rheumatoid arthritis, psoriasis and uveitis during pregnancy.

**Pregnancy and Neonatal Outcomes in MS**

Accumulating evidence suggests that women with MS are not at a higher risk for pregnancy and neonatal complications than healthy women. Any differences observed may be due to cultural, religious, geographical and regional variations. A Meta-analysis performed in 2011 was the first to report aggregate outcomes from 22 papers published since 1983 (n=13,144 women with MS). The findings from this meta-analysis for each of the following outcomes are summarized below.

a) **Abortion rates**

Abortion rates of 20-30% from both miscarriage and elective terminations have been reported in MS pregnancies.
b) Delivery Mode
Rates of cesarean sections among women diagnosed with MS vary widely depending on geographical region (9.6%-41.1%).

c) Adverse Neonatal Outcomes
About 10% of children born to women with MS are born prematurely (<37 weeks at birth) regardless of the geographical region in which the children are born. Low birth weight (<2500 grams/birth) occurs in nearly 6% of MS pregnancies. Non-specific malformations and neonatal death were reported in 3.03% of 1081 MS deliveries, none of which appeared to be disease or therapy related.

d) Birth Weight
Was not a primary outcome in this meta-analysis. In some studies however, infants born to mothers with MS had lower s than infants born to healthy mothers, although the difference didn’t appear to be clinically significant. Many factors impact infant . Racial and geographical differences are known to play a role. A Norwegian study found a reduction in in babies born to mothers with established MS compared to those prior to MS onset/diagnosis. Reasons for lower have not be clarified however it is postulated that it may be due to a neurologically driven compromised uterine environment due to MS and/or altered maternal immune functions.

Disease Modifying Drugs (DMD)
“Disease Modifying Drug” (DMD) or Disease Modifying Therapy (DMT) is the terminology used to describe medications used to treat MS. These therapies are designed to modify disease course by reducing frequency and severity of relapses. Most DMDs are approved for use throughout the relapsing-remitting phase of multiple sclerosis. Due to significant cost, inconvenience, anxiety associated with self-injections (many first line
therapies are injectable), and lack of confidence in therapeutic potential as well as cognitive and physical side effects, low therapy adherence is an ongoing challenge.  

Acute relapses are treated with steroids, typically a combination of high dose oral prednisone with or without taper and/or IV methyl-prednisolone. Steroids are quite effective in treating relapse symptoms within a few days, however chronic administration of high dose steroids are associated with side effects such as osteoporosis, immunosuppression, hypertension, hyperglycemia, impairment in wound healing, metabolic disturbances, glaucoma and cataracts, as well as cognitive, psychiatric and behavioral disturbances.  

**Safety of DMDs in Pregnancy**

The study by Hughes et al. was the first to suggest that DMD usage within 2 years of conception is associated with a 45% reduction in early postpartum relapses.  In contrast DMD use during pregnancy does not seem to affect risk of postpartum relapse.  As pre-pregnancy relapses are predictive of disease activity postpartum, reducing relapses through DMDs prior to conception has and will continue to be a common healthcare strategy in controlling disease, leading to more unexpected and/or intentional exposures in pregnancies. Studies regarding safety of exposures to DMDs have been conducted, although data is limited for the second generation class of DMDs.

**First Line Disease Modifying Drugs**

[Glatiramer Acetate (Copaxone®), Interferon Beta-1a Avonex®, Rebif®), Interferon Beta-1b (Betaferon®)]

The drugs in this class are the oldest line of DMDs used to treat MS, and large number of exposures demonstrated no increase risk to date in
teratogenicity, spontaneous abortions, preterm birth nor other adverse pregnancy and neonatal outcomes. Over 500 human exposures to glatiramer acetate along with favorable outcomes in animal studies make this the most favorable DMD for planning and pregnant women with MS.

Interferon Beta-1a/b (INFB-1a/b) were the first DMD introduced and have the most number of pregnancy exposures reported of all DMDs. 1045 exposures to INFB-1b with 423 known outcomes, and 1022 INFB-1a exposures with 679 known outcomes, demonstrated the relative safety of these drugs. Previous studies in primates at doses 40 times the human dose did show a higher rate of spontaneous abortions, and because of this, some healthcare providers prefer to discontinue INFBs 1-2 months prior to conception.

Both glatiramer acetate and interferons are very large molecules and are unlikely to transfer readily into breastmilk, rendering them as acceptable choices in the postpartum period as well.

**Orals and Second Line Disease Modifying Drugs**

**Oral Disease Modifying Drugs**

[Fingolimod (Gilenya®), Dimethyl Fumerate (Tecfidera®), Teriflunomide (Aubagio®)]

**Fingolimod (Gilenya®)**

is the first oral DMD approved for MS. Animal studies demonstrated neural tube defects in mice deficient in the converting enzyme, and miscarriages at doses lower than the human therapeutic dose. sixty-six pregnancies with known exposures to this agent were reported in a 2014 paper, of which 5 (7.6%) had abnormal fetal development in both terminated pregnancies and live births. A
higher rate of spontaneous abortions (24%) was also reported.\textsuperscript{53} Currently, it is strongly recommended that fingolimod be discontinued at least 2 months prior to conception and that necessary measures be taken to prevent pregnancies in women of reproductive age on this drug.\textsuperscript{53} The drug is also excreted in human milk and has oral bioavailability, hence breastfeeding is also discouraged in postpartum women on this drug.\textsuperscript{50}

**Dimethyl Fumarate (Tecfidera®)**

Delayed release dimethyl fumarate is an oral agent specifically used to treat MS.\textsuperscript{54} Animal studies have demonstrated transfer of this agent through the placenta, with high dose administration (up to 16 times the human dose) leading to adverse outcomes such as delayed sexual maturation, reduced testicular weight, increase in spontaneous abortions, lower fetal weight and delayed ossification.\textsuperscript{50} Knowledge regarding exposure in human pregnancies is limited. One post-marketing study of 30 pregnancies with known outcomes reported 10 live births, 13 miscarriages, 2 ectopic pregnancies and 5 elective terminations.\textsuperscript{54} Currently available data does not suggest an increased risk for teratogenicity and miscarriage in human pregnancies, and given the extremely short half-life of the drug, it can be discontinued when the patient becomes aware of pregnancy.\textsuperscript{54} Due to the limited amount of data, the drug should only be used when risk outweighs its benefits, and proper contraceptive measures must be taken.

**Teriflunomide (Aubagio®)**

is a category X drug due to observed teratogenic effects and embryo-fetal loss in exposed rats and rabbits.\textsuperscript{50} The adverse outcomes may have partially been due to maternal toxicity.\textsuperscript{50} In humans the data is limited. A study by Kieseier et al. reported outcomes in 70 pregnancies with known teriflunomide exposures. They reported 26 live births, 13 miscarriages, 29 terminations and 2 unknown outcomes.\textsuperscript{55} All live births appeared healthy; the spontaneous abortion rate was 18.6%,
which was not significantly different from general population rates.\textsuperscript{55} It must be noted that the duration of exposure was very short, and most women with live births (N=23) underwent an accelerated elimination process with cholestyramine or activated charcoal, which is recommended when pregnancy is suspected in women on this drug. Given the sustained teratogenic effects in animals and scarcity of human data, women on this drug are strongly advised to use proper contraceptives. The drug is found to transfer into breastmilk in animals and should be avoided during lactation.\textsuperscript{50}

Alemtuzumab (Lemtrada®, Campath®)

is administered initially as a 5 day infusion, followed by a single 3 day infusion 12 months later and does not require additional dosing. Higher fetal loss and decreased fetal B and T lymphocyte populations have been observed in animal studies.\textsuperscript{50} The 12 day long biological half-life necessitates pregnancies to be avoided within 4 months of receiving this agent.\textsuperscript{50} In a 2014 abstract presented at the joint American Committee For Treatment and Research in Multiple Sclerosis (ACTRIMS)-European Committee For Treatment and Research in Multiple Sclerosis (ECTRIMS) meeting in Boston, Massachusetts, of the 139 pregnancies in women on this drug, there were 67 live births, 14 elective terminations, 24 miscarriages, 1 stillbirth and 33 unknown outcomes.\textsuperscript{56} Only six pregnancies were conceived within 4 months of discontinuation. The rates of malformation and spontaneous abortion were not increased in this case series. One thyrotoxic event occurred in a 21 day-old infant, which resolved after treatment. Both pregnancy and breastfeeding should be avoided in women on this drug until further studies confirm its safety.

Rituximab (Rituxan®)

is a chimeric monoclonal antibody. Animal studies have revealed a dose dependent decrease in lymphoid B-cells when administered to pregnant cynomolgus monkeys, peripheral B-cell depletion and immunosuppression
in offspring at birth with eventual recovery at 6 months of age.\textsuperscript{57} Of the 153 human pregnancies with known outcomes exposed to this drug there were 90 live births, 33 spontaneous abortions, one stillbirth due to fetal hypoxemia from umbilical cold knot, 28 elective terminations and one case of maternal death due to cerebral hemorrhage with underlying idiopathic thrombocytopenic purpura.\textsuperscript{57} The rate of malformation was not increased in this cohort, and of the 90 live births 68 were full-term deliveries. 22 were born prior to 37 weeks with no extreme prematurity reported. On neonate died at six weeks from unknown causes. It is important to note that only two of these exposures were for an MS indication, and the rest were treated for other autoimmune conditions in varying severity such as lupus. Despite the apparent lack of teratogenicity, hematologic abnormalities such as B-cell depletion, thrombocytopenia, granulocytopenia and anemia in babies have been reported and until further evidence becomes available, pregnancy and breastfeeding should be avoided while on this medication.\textsuperscript{50}

\textit{Natalizumab (Tysabri®)}

Chapter 2 of this thesis has been devoted to gestational exposure to this drug. Therefore, this drug will be discussed in greater detail than other DMDs.

This drug is typically approved as a second line therapy for patients with RRMS that have failed to respond to other DMDs, or as first-line for patients with very active disease at onset. It is administered as a 300 mg IV infusion once every four weeks, and costs around $40,000/year in Canada.\textsuperscript{58}

Natalizumab is a humanized monoclonal antibody which binds to the $\alpha_4$
subunits of $\alpha_4\beta_1$ & $\alpha_4\beta_7$ integrin molecules. These integrin molecules known as Very Late Antigen-4 (VLA-4), are integrin dimers expressed on the plasma membrane of leukocytes. When these leukocytes are activated by chemotactic agents and other stimuli, the dimers undergo conformational changes and bind with high affinity to their receptors on Vascular Cell Adhesion Molecules-1 (VCAM-1). VCAM-1 are expressed on endothelial cells of brain and spinal blood vessels and once bound, the VLA-4 is able to penetrate these cells. In MS they cross the blood-brain barrier (BBB) in this way, leading to inflammation at the site of disruption. Natalizumab binds to the $\alpha_4$ subunits of these integrin dimers and prevent the binding to VCAM-1 and subsequent lymphocyte migration across the BBB.

It has demonstrated great efficacy in several clinical trials. In the phase III AFFIRM Trial (Natalizumab Safety and Efficacy in RRMS), a 2 year follow-up of 856 patients with EDSS scores (0-5) and one relapse in the preceding 12 months, who had been DMD naïve for at least a year, a 68% reduction in annualized relapse rates, and a 42% reduction in disability progression, 83% reduction in new and emerging T2-hyperintense MRI lesions, and a 92% reduction in gadolinium enhancing lesions were observed. Brain atrophy was significantly decreased in the second year of treatment, and the probability of individuals being free of disease activity was 16 fold greater than placebo treated groups. Overall, patients with more severe disease appeared to benefit more from the treatment; those with EDSS $\geq$2 were more likely to experience confirmed disability improvement over the 12 week trial period. Beneficial effects on visual function as well
as quality of life were also reported in the post-hoc analysis of this trial.\textsuperscript{61}

In the two-year SENTINEL (Safety and Efficacy of Natalizumab in Combination with Avonex in Patients with RRMS) RCT, 1196 patients already receiving weekly interferon β-1a were randomized to add either natalizumab or placebo to their preexisting treatment.\textsuperscript{62} Patients had EDSS scores 0-5 and had experienced at least one relapse in the last 12 months while treated with INFB-1a. Patients in the natalizumab arm had a 55% reduction in annualized relapse rates compared to placebo arm, with an 83% reduction in T2 MRI lesions, and a 24% reduction in relative risk of sustained disability progression.\textsuperscript{61}

In the phase II trial GLANCE (Glatiramer Acetate and Natalizumab Combination Evaluation), 110 patients on glatiramer acetate (GA) for at least 12 months, with EDSS scores 0-5, experiencing at least one relapse while on GA therapy, were randomized to receive placebo or natalizumab as an add-on therapy for 24 weeks.\textsuperscript{63} The combination therapy with natalizumab proved superior to GA alone, with 74% fewer gadolinium enhancing lesions and 61% reduction in new or enlarging T2-hyperintense lesions.\textsuperscript{63}

Natalizumab was approved by the FDA in 2004 for RRMS. In 2005, the FDA recommended suspension of the drug due to the emergence of cases with Progressive Multifocal Leukoencephalopathy (PML) in treated individuals.\textsuperscript{61} PML is a rare and often fatal viral disease, caused by the reactivation of John Cunningham virus (JCV). JCV is latent in different tissues, including the kidney, bone marrow and lymphoid tissue.\textsuperscript{64}
Approximately 60%-70% of adults test positive for serum antibodies to this virus, and it is not pathogenic in the general population. However in immunocompromised individuals (i.e. HIV, AIDS, SLE, or those on immunosuppressant drugs), the previously dormant virus may become re-activated, attacking oligodendrocytes in the brain.\textsuperscript{64,65} It can cause demyelination, and may lead to severe disability and death.\textsuperscript{65} Risk factors associated with JCV re-activation in natalizumab patients include: (1) prior usage or concomitant use of other immunosuppressive drugs; and (2) the number of infusions of natalizumab.\textsuperscript{61} Risk of JCV infection increases considerably after the 24\textsuperscript{th} infusion, from 0.19/1000 to 1.37/1000 in immunosuppressant naïve patients and from 0.4/1000 to 10/1000 in patients that had prior exposure to immune-suppressants.\textsuperscript{61,64} Titters of serum anti-JCV antibodies have been used to create a “JCV index”, which allows for more refined risk- stratification of patients.\textsuperscript{64} PML risk is low (0.1-0.6/1000) at an anti-JVC antibody index of <0.9 but increases substantially in patients with index values above 1.5 who have received more than 24 infusions of natalizumab (0.9-10/1000 patients).\textsuperscript{64} Mortality rate is 30-50\% for all causes of PML, and even with survival, permanent disability is likely.\textsuperscript{65} An 18\% rate of mortality has been reported in natalizumab associated PML. The first three PML cases in natalizumab-exposed patients from the SENTINEL trial, with the subsequent death of one of these patients, lead to the voluntarily suspension of the drug.\textsuperscript{59} The FDA mandated suspension of natalizumab was lifted in 2006, at which point careful monitoring systems, including the TOUCH (Tysabri Outreach:
Unified Commitment to Health Prescribing Programs) and TYGRIS-ROW (Tysabri Global Observational Program in Safety-Rest of the World) programs, were introduced, for US and Global patients respectively. The purpose of these programs was to monitor and minimize risk in patients enrolled, by restricting prescription and dispensing to certain pharmacies and patients at specific sites.\textsuperscript{64} All patients initiating therapy with natalizumab must enroll in one of these programs.

As of May 31-2017, 170,900 patients worldwide had received this drug in the post-marketing setting, and the global overall incidence of PML was 4.21/1000 (95%CI: 3.91-4.52 per 1000 patient).\textsuperscript{66} As of June 6, 2017 there have been 731 cases of PML reported in association with natalizumab (728 with MS and three with Crohn’s).\textsuperscript{66}

Discontinuation or interruption of natalizumab has been associated with disease resurgence, or rebound, sometimes more severe than the pre-treatment period.\textsuperscript{64} In a meta-analysis of 1866 patients receiving natalizumab, 544 discontinued without alternative DMD substitution. MS activity peaked 4-7 months after discontinuation in this cohort.\textsuperscript{64} Up to 58% of discontinuers experienced disease resurgence with an annualized relapse rate up to four-fold higher than the pre-natalizumab period.\textsuperscript{64} Switching to another first or second line disease modifying drug upon natalizumab discontinuation may increase the chance for remaining disease activity free.\textsuperscript{64}
Natalizumab in Pregnancy

In animal studies, high doses of natalizumab were associated with a reversible decrease in fertility, and during gestation, with reduced survival, anemia and thrombocytopenia in offspring.50

A few small case series of women taking natalizumab during pregnancy reported normal pregnancy outcomes, with no clear teratogenic effects. 67,68 One small case series found mild hematological alterations in 10 out of 13 children exposed in the third trimester of pregnancy, but these were transient and did not lead to functional limitations. Overall, the infants were healthy.69

The Tysabri Pregnancy Exposure Registry reported outcomes in 355 patients.70 73 discontinued prior to conception, 275 in the first trimester, 10 in the second trimester, 1 in the third trimester. Only 4 women took the medication throughout pregnancy. There were 316 (87.1%) live births, 1 stillbirth, 14 (3.9%) elective terminations, and 32 (8.8%) spontaneous abortions. The rates of major birth defects were 5.1%, slightly above the general population risk. However, no pattern for defects was observed. Overall, no increased risk for any adverse outcomes was noted in this registry.

Our study, published in 2015, was the first to report outcomes in 101 MS patients exposed to natalizumab, while using disease matched and healthy comparison groups. By incorporating a disease-matched comparator we were able to segregate disease impact from natalizumab impact on pregnancy and neonatal outcomes.71
The paper is presented as the second chapter of this thesis.

**Other Monoclonal Antibodies**

The newer monoclonal DMDs have limited published data on human pregnancy exposure. Women on these medications are advised to use proper protection to prevent pregnancies. Below is a summary of inadvertent exposures across trials and in clinical practice.

**Ocrelizumab (Ocrevus ®)**

is an anti-CD20 marker on B lymphocytes. A poster presented in the 2017 Annual Meeting of the Consortium of Multiple Sclerosis Centers in New Orleans, reports 48 human pregnancy exposures to ocrelizumab.\(^{72}\) 15 of these exposures occurred in MS patients, 10 in SLE, and 21 in Rheumatoid Arthritis. Only seven of the 15 MS pregnancies had confirmed fetal exposure to the drug. 4 of the seven pregnancies were electively terminated, 2 pregnancies were ongoing, and one healthy term baby was delivered. Outcome of pregnancy in the SLE and RA groups have not yet been reported.

**Daclizumab (Zinbryta ®)**

is a humanized monoclonal antibody targeting CD25. Pregnancy outcomes across several trials of daclizumab have been reported by Gold et al in a 2016 paper.\(^{73}\) A total of 36 women receiving at least one dose of daclizumab within six months of conception reported 38 pregnancies. 20 live births, 4 miscarriages, 8 elective terminations, and 2 ectopic pregnancies occurred in this cohort. Two of the pregnancies were lost to follow-up, and two
pregnancies were ongoing at the time of publication. One congenital heart defect (complex transposition of great vessels) was observed in live births.

**Ustekinumab (Stelara ®)**

is a monoclonal antibody blocking the IL-12/23 mediated inflammatory pathways. Only seven cases of human pregnancy exposures to the drug have been reported. Severe psoriasis was the indication for therapy in six of the women and Crohn’s and psoriasis for one. There were six normal pregnancy outcomes reported, and one miscarriage.\(^{74-79}\)

**Vitamin D Status in Pregnancy**

Vitamin D status is determined by measuring serum calcidiol [25(OH)D], which reflects total Vitamin D\(_3\) & D\(_2\) from all sources.\(^{21}\) To date no consensus exists on an “optimal” level. However majority of experts agree that serum levels of at least 50 nmol/L must be attained in both general and pregnant populations. Levels below 50 nmol/L is classified as insufficient and less than 37.5 nmol/L as deficient by most experts.\(^{21}\) More recently, serum calcidiol levels above 80 and 100 nmol/L have demonstrated the most immunological and bone protection.\(^{21}\) A recent RCT demonstrated that maternal calcidiol levels of 100nmol/L is required in order to optimize renal and placental production of [1,25 (OH)\(_2\)D] hormone.\(^{22}\) These findings, have challenged the widely accepted threshold of “<50nmol/L” to define insufficiency, favoring higher cutoff levels in pregnancy.

Significant differences in Vitamin D metabolism in pregnant versus non
pregnant states exist. While conversion of Vitamin D to calcidiol does not appear to change substantially in these two states, the conversion of calcidiol to calcitriol is several times higher. By the 12th week of gestation, calcitriol levels are two times higher than in non-pregnant adults, and 2-3 fold higher by the third trimester. Although VDBP rises in parallel to calcitriol, it is the free fraction of calcitriol that continues to rise. The rise in this active metabolite is thought to be due to enhanced renal production of calcitriol, in addition to contributions from the placenta.

In line with this, calcitonin and prolactin levels which further stimulate renal 1-alpha-hydroxylase also increase in pregnancy. This phenomenon is unique to pregnancy alone and is not seen in the lactation phase, suggesting a regulatory role of Vitamin D in both innate and acquired immune response on the fetal-maternal interface. One theory is that the maternal tolerance of the half foreign fetus is dependent on increased levels of calcitriol. Calcitriol levels as high as 700pmol/L in pregnancy without adverse effects, (a level which can induce hypercalcemia in the non-pregnant state) seem to support this immunological tolerance theory.

**Gestational Vitamin D Deficiency and Neonatal Outcomes**

Vitamin D Deficiency (VDD) is a common health issue prevalent among children and adults of all age groups worldwide. Pregnancy is a known risk factor for hypovitaminosis D, particularly in individuals with darker skin. One study revealed deficiency in 29% and 5%, and insufficiency in
54% & 47% of African descent Americans vs Caucasian Americans respectively. A study from Netherlands reported VDD within 40-94% of different ethnic cohorts of pregnant women residing in Netherlands who had immigrated from Asia, Iran, India, Pakistan, Turkey, Somalia, UK, Ireland, as well as darker skinned and veiled women. VDD prevalence is highest in the winter months, and also in higher latitudes. Maternal VDD has been associated with several adverse maternal & neonatal outcomes. Women with VDD have been reported to have a higher risk for pre-eclampsia, gestational diabetes, higher rates of cesarean section and other health problems including immune dysfunction, poor placental implantation, higher bone turnover, bone-loss, osteomalacia and myopathy. Neonatal outcomes have been associated with maternal calcidiol status. Preterm birth, low birth length and head circumference, postnatal growth, immune response (both adaptive and innate) have been associated with gestational maternal serum calcidiol levels. Severe maternal VDD has been implicated in neonatal seizures and rickets at birth. More recently, the role of Vitamin D in the immune system has gained attention. It is now known that both calcitriol and calcidiol are able to induce the expression of cathelicidin (LL-37) (an endogenous antimicrobial peptide generated in response to microbial invasion) in monocytes and macrophages. Low serum calcidiol levels are associated with inefficient cathelicidin mRNA induction, and supplementing with Vitamin D can reverse this inefficiency. It has also been shown that INF-γ mediated antimicrobial actions of macrophages is Vitamin D dependent. Hence maternal VDD and
the subsequent deficiency in the neonate, increases the risk for infection, especially respiratory infections. Studies have found a higher rate of respiratory syncytial virus (RSV) bronchiolitis in the first year of life in neonates born with VDD; a six-fold greater risk in neonates with cord serum calcidiol levels <50 nmol/L versus 75 nmol/L or higher has been reported.\textsuperscript{22} A higher risk for lower respiratory tract infections in the first five years of life has also been observed.\textsuperscript{22} Vitamin D also plays a role in adaptive immunity.

VDR are found on activated T and B lymphocytes, and in the presence of calcitriol there is subduing of pro-inflammatory response.\textsuperscript{22} Hence calcitriol is involved in the activation of innate immunity in response to infections, while inhibiting proliferation and differentiation of immune cells in adaptive immunity, promoting an anti-inflammatory state. It is not surprising to find a link between Vitamin D and both autoimmune diseases, including MS, and certain cancers in offspring.\textsuperscript{22}

In a Finnish study with 176 MS women, first trimester calcidiol levels were significantly lower in MS mothers than controls, and the subsequent MS risk in offspring of these women were two times higher.\textsuperscript{81}

\textit{Vitamin D Intake and Birth Weight}

Total intake of Vitamin D has been shown to be a significant predictor of birth weight even after adjusting for caloric intake, use of calcium, iron, folate, protein, zinc, and gestational length.\textsuperscript{80} This may be attained through
increased rates of bone mineral buildup in the developing fetus. Higher third trimester maternal serum calcidiol and additional supplementation with Vitamin D have been associated with greater postnatal somatic growth and increased lumbar spine bone mineral content in babies. In three trials with over 460 women receiving Vitamin D during pregnancy a reduced risk for low birth weight babies (<2500 gram) was reported. Head circumference was also significantly higher in neonates born to mothers with higher calcidiol levels. Another study reported head circumference at three and six months postpartum to be correlated to cord blood calcidiol levels at birth. It is well known that maternal serum calcidiol levels in pregnancy and neonatal cord blood levels are highly positively correlated ($r^2=0.81, p$-value<0.001). An 11 gram increase in birth weight for every 40 IU of maternal Vitamin D intake has been reported. One Canadian study demonstrated that restricting maternal milk consumption was associated with an 80 gram lower birth weight compared to non-restricted intake, and both milk & Vitamin D intake were significant predictors of birth weight. A 2015 meta-analysis of 13 RCT trials looking at the effect of maternal Vitamin D supplementation on birth outcomes found an increase in birth weight (107 grams) and a greater length at birth (0.3cm) in eight and six of the supplemented groups in these trials respectively. In the same meta-analysis, low birth weight, small for gestational age babies and preterm births did not differ significantly from controls. However several limitations in the RCTs included in the analysis were reported to have impacted the findings, including heterogeneity of the cohorts, type,
duration and timing of Vitamin D supplementation, and differences in defining maternal and neonatal endpoints.

Overall response to maternal supplementation varies widely, and birth weight differences range from 60-410 grams.\textsuperscript{22,80} This range of response to supplementation may be attributed to many maternal and neonatal factors, as well as the small sample sizes and methodological limitations in these studies.

**Safety of Vitamin D Supplementation in Pregnancy**

Although some Vitamin D-induced teratogenic potential has been reported in offspring of rats and rabbits, data in humans has been reassuring.\textsuperscript{84} Maternal doses as high as 5 mg, equivalent to 200,000 IU/day, has not been associated with any negative outcomes.\textsuperscript{84} It appears that as long as maternal serum calcidiol levels are within normal range, risk to fetus is unlikely. Maternal toxicity however due to chronic overdose may adversely impact the fetus.\textsuperscript{84} The threshold for toxicity has not been established, but it is possible that regular exposure exceeding 10,000IU/day or a single dose exceeding 300,000IU may be harmful.\textsuperscript{21}

As mentioned earlier, we still lack a definition for “optimal” serum levels of calcidiol in pregnant and non-pregnant populations. As serum levels between 75-100nmol/L gain favorable attention in the scientific community, it has been suggested that a daily Vitamin D dose of 1000 to 1600 IU per day may achieve these levels in non-pregnant individuals.\textsuperscript{21} Daily doses within this range were shown to increase serum calcidiol levels by 1.2 nmol/L for every
40 IU when baseline levels were low, and smaller increments were observed when baseline levels were higher.\textsuperscript{21}

In one study, supplementation with 1000 IU/day increased both maternal and cord serum levels by 12.5-15 nmol/L.\textsuperscript{85} This is significant, as it implies deficiency in neonates can be effectively prevented through maternal supplementation during pregnancy.\textsuperscript{85} At term, maternal levels of 80 nmol/L may be required to yield 50 nmol/L in cord blood.\textsuperscript{85} While many studies have demonstrated that Vitamin D supplementation increases maternal serum calcidiol levels, response to supplementation is highly variable and unpredictable. In a meta-analysis of 414 women across 4 trials, an increase in range of 11 to 152 nmol/L was reported.\textsuperscript{21} Thus the rate and level of a safe Vitamin D increase in pregnant women is unknown, especially in women with sufficient Vitamin D levels.

Intake of 6000 IU/day has been suggested after 400 IU/day and 800-1600 IU/day failed to effectively increase maternal circulating calcidiol levels.\textsuperscript{80} A daily dose may also be superior at achieving higher serum concentrations at the end of the pregnancy than a single high dose.\textsuperscript{21} Many over-the-counter prenatal vitamins sold in North America contain 400 IU of Vitamin D, which is deemed inadequate by many experts.\textsuperscript{22} A randomized controlled trial, with more than 500 women of diverse backgrounds, demonstrated that 4000 IU/day in the second trimester is superior to 2000 IU/day in attaining serum calcidiol levels of 100 nmol/L.\textsuperscript{86} No significant adverse events were seen in the higher dose group, and more importantly, pregnancy-related comorbidities were significantly higher in the lower dose groups.
While sun exposure is the easiest method to substantially increase serum calcidiol levels (10-15 minutes of whole body exposure can produce 10,000-15,000 IU within 24 hours), the risk for skin cancer, inadequate UVB in winter and fall, especially in higher latitudes, make this an unreliable method. Dietary sources can contribute to calcidiol status, however studies show that dietary intake is inadequate. A study from one of the poorest cities in New Jersey revealed dietary intake of Vitamin D to be associated with parity, pre-pregnancy BMI and ethnicity. In this study nulliparous women and women with lower BMI had higher Vitamin D intake, while minority women (Hispanics and Blacks) and overweight and obese women had a lower intake. The dietary contribution in this cohort was an estimated 192 IU/day. While poverty and lack of education may have been reasons for low dietary Vitamin D intake, a study of a more affluent and well educated cohort, also demonstrated low total intake equating to approximately 500 IU/day (from diet and supplements). Other studies have reported a 10% contribution to serum levels from dietary sources. Hence supplementation is the most practical, reliable, and inexpensive method to improve Vitamin D status in pregnancy.

**Vitamin D and Breastfeeding**

Breastmilk contains no Vitamin D of its own, but 20% of maternal Vitamin D can enter breast milk. Hence maternal supplementation with Vitamin D in the postpartum period is an infant’s only food source of Vitamin D. Thus babies that are born with deficiency will continue to be deficient in the
postpartum period, and if the mother is also deficient, she will fail to provide her breastfeeding infant with adequate Vitamin D. Unlike pregnancy, the parent compound Vitamin D itself (not calcidiol) directly transfers into milk. Given the shorter half-life of Vitamin D (12-24 hours), a daily intake of Vitamin D is required in breastfeeding women to ensure adequate levels. Since only 20% of maternal Vitamin D will transfer into milk, a lactating woman may require a higher dose of Vitamin D intake than during her pregnancy to yield 400 IU/day in breastmilk. In one study 4000IU/day was not enough to achieve this level in breastmilk. Based on pharmacokinetic studies, 6000 IU/day may be necessary.

**MS, Pregnancy and Vitamin D**

As stated earlier, both pregnancy and breastfeeding are risk factors for Vitamin D insufficiency and deficiency. Given that in MS lower serum calcidiol has been associated with increased disease activity, the impact of supplementation on postpartum disease course and neonatal outcomes is of particular interest.

In a 2015 study by Jalkanen et al. serum calcidiol levels were compared between a pregnant MS, and a healthy control group. 15 Finnish women with RRMS and 6 healthy women provided a series of blood samples during pregnancy and in the postpartum period. Serum calcidiol levels in MS patients were significantly lower throughout pregnancy, and reached a nadir in the first month postpartum. Third trimester levels were 47 vs. 63 nmol/L while levels in the first month postpartum were 37 & 53 nmol/L in
MS and controls respectively. Levels were below 50 nmol/L in 73% and 80% of MS patients during pregnancy and postpartum respectively. Patients who were relapse free in the pre-pregnancy year had higher serum calcidiol levels in pregnancy and postpartum, although no direct link was observed between serum levels and postpartum relapses. As data on serum calcidiol levels in MS pregnancies is scarce, this small study provides important preliminary evidence regarding the vulnerability of pregnant women with MS to Vitamin D insufficiency compared to healthy women, and the importance of supplementation to correct these deficiencies. In a small study by Gould et al, 28 women with RRMS were followed up in the postpartum period. The objective was to examine the role of serum calcidiol levels in pregnancy and lactation and risk for MS relapse. 93% of this cohort consisted of Caucasian women and all but one supplemented with prenatal vitamins. Serum calcidiol was measured in the third trimester, and again at two, four and six months postpartum. 50% of this cohort breastfed exclusively. Third trimester levels and exclusive breastfeeding were strongly associated with serum calcidiol levels, and by four and six months postpartum, the serum calcidiol levels were lower by 11 nmol/L in the exclusive breastfeeding women which was significant. 71% of the total cohort experienced at least one relapse in the first 12 months, and serum calcidiol levels were higher in those who relapsed. Interestingly, a higher proportion of exclusively breastfeeding women, who consequently had lower serum calcidiol levels, remained relapse free. This is one of the first studies to suggest that Vitamin D levels in isolation may not predict postpartum
relapse; exclusive breastfeeding and the hormonal shifts it promotes, may confer a protective effect that offsets insufficient Vitamin D levels.

A 2014 study by Runia et al. examined the role of serum calcidiol levels on postpartum relapses and quality of life in women with MS.\textsuperscript{90} 43 women with RRMS from Rotterdam, and 21 healthy controls were used for this study. No differences in baseline serum calcidiol levels or change in calcidiol levels over the course of pregnancy and postpartum period were observed between patients and controls. Serum calcidiol levels peaked in the third trimester and dropped significantly thereafter in both groups. The between group difference was not significant, with a nearly identical pattern in both groups. In controls only, higher calcidiol levels were correlated with better scores on several quality of life measures including general health, mental health and social functioning. Like Gould’s study\textsuperscript{89} there was no association between serum calcidiol levels and postpartum relapses, but unlike Gould’s study there was no association between breastfeeding and serum calcidiol levels. Notably in this study, distinction was not made between breastfeeding and exclusive breastfeeding. Study limitations include the small size, the unknown dose of Vitamin D in prenatal supplements that were used in 42\% of the cohort, unknown prenatal use status in 13\% of the cohort, and breastfeeding details. Furthermore the serum calcidiol levels appeared to be sufficient (>60 nmol/L) throughout the study period in both groups. This suggests selection bias in the population — as previously discussed, calcidiol levels are low in the majority of healthy women and those with MS. Furthermore, without a disease matched group
with lower serum calcidiol levels, the researchers could not investigate any protective effect attributable to Vitamin D.

The impact of supplementation in MS pregnancies is understudied. The first RCT published on this topic was a small 2015 open-label phase I/II trial in an Iranian cohort consisting of 15 patients with MS. Six of the patients were randomized to receive 50,000 IU of Vitamin D once a week, starting at 12-16 weeks of gestation; the remaining nine patients only received routine care and no supplementation. Both groups were followed up every 8 weeks until 6 months postpartum and information on EDSS and relapses were collected. Baseline serum calcidiol levels were low: 39 and 47 nmol/L in treated and control groups respectively. In the treatment group serum levels reached 86 nmol/L at six months postpartum while it continued to decline in the controls and fell to 37 nmol/L. EDSS scores did not change in the treated group and were significantly lower than controls; the same was observed in postpartum relapses. Pregnancy outcomes and breastfeeding status were not reported in this study. It was not clear how the deficiency was addressed in the control group. The sample size was very small, and several other limitations exist in this study. Nevertheless, as the first study to examine moderately high Vitamin D supplementation in MS pregnancies, it provides a platform for future research. No adverse events were reported in the high dose group and the apparent favorable disease outcomes, recapitulates the safety of higher doses given in non-pregnant adults with MS.
Statement of the problem

MS impacts more women than men, and most commonly manifests during the reproductive years. Since pregnancy is not expected to worsen disease course, and may even induce disease remission, many women with MS plan pregnancies and have children. However, remission during pregnancy is not guaranteed and conception may be delayed. The scarcity of data on the gestational safety of DMDs, pressures planning women to forgo treatment to become pregnant. This puts women with good control of disease using DMDs, at risk of disease resurgence in the months leading to conception and even during pregnancy. Many of these women experience tremendous angst and obscurity when strategizing therapy during this stage of their lives. When experiencing acute relapses typical treatment necessitates high dose steroids which carry their own risk to the developing fetus. Furthermore, breastfeeding coincides with postpartum disease flare-up. Again, little is known about the safety of most DMDs in lactation. Thus today women with MS continue to struggle with making the best treatment choices for controlling disease while protecting their unborn/breastfeeding infant.

Rationale

In this thesis we address the safety of gestational exposure to natalizumab in manuscript #1, and high dose Vitamin D in manuscript #2. The efficacy of Vitamin D supplementation for preventing MS relapses in the postpartum period is examined in manuscript #3 by comparing postpartum disease resurgence in two different MS cohorts, one with and the other
without Vitamin D supplementation.

Natalizumab is a second line DMD shown to be highly effective in minimizing disease activity. Women with MS on natalizumab tend to have excellent control of disease and may prefer to continue treatment until conception occurs. However, given the scarcity of safety data on this drug in pregnancy, most women experience anxiety when determining the best time to discontinue treatment. We identified a German cohort of pregnant women with natalizumab exposure in early pregnancy and compared their pregnancy and neonatal outcomes to a healthy cohort as well as a disease-matched cohort exposed to other DMDs. Reporting outcomes in this cohort and comparing them to a disease-matched group, provides an informative source for future mothers with pregnancies exposed to this medication.

The popularity of Vitamin D is on a rise in patients with MS, and we have identified a group of Canadian women that supplement regularly from the time of their disease diagnosis. Currently no optimal dose of Vitamin D supplementation, nor optimal serum calcidiol levels in MS patients have been established, and regular serum monitoring in those that supplement is not implemented. Furthermore many of these women identified exceed the maximum 4000IU/day Vitamin D intake recommended by Health Canada. Pregnancy in these patients add another dimension of complexity, as agreement regarding optimal maternal dose and serum levels during gestation, even in healthy women has not yet been reached. Whether supplementation leads to better disease outcomes, particularly in the postpartum period needs to be investigated. If justified, it has the potential
to become an innovative and inexpensive strategy in delaying disease activity, affording women the ability to breastfeed for a longer duration, benefitting both mother and infant. To address this question, in the second manuscript we compare postpartum disease outcomes in our Canadian women to a disease matched group of German women that do not supplement with Vitamin D nor take any prenatal vitamins.

Finally, as with any effective therapy, use during pregnancy needs to be supported by assessing neonatal outcomes following that exposure. In the third manuscript we report pregnancy and neonatal outcomes in our Canadian cohort by comparing women that exceed the recommended 4000 IU/day Vitamin D intake to those that take lower doses. This will provide a stage for future studies to investigate optimal dosing and serum levels of Vitamin D yielding the most benefit to mothers with MS and their breastfeeding infants.
CHAPTER 2
MANUSCRIPT ONE

Pregnancy Outcomes Following Natalizumab Exposure. A Prospective Controlled Observational Study

Journal Reference


Abstract: 190 words

Title: 95 characters with space

Tables: 4 Figures: 0 References: 19

Keywords

Multiple Sclerosis; 2. Pregnancy Exposure; 3. Natalizumab; 4. Fetal Safety

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Funding

The German MS and Pregnancy Registry is partly supported by Biogen Idec, Bayer, Novartis Teva, Merck, and Sanofi. The Motherisk Nausea and Vomiting of Pregnancy counseling line is partially
funded by Duchesnay Inc.

Authors Contribution

Neda Ebrahimi, M.Sc.: Study concept and design, data acquisition, analysis, interpretation, drafting and revision of manuscript.

Sandra Herbstrott M.Sc.: study design, data acquisition, and analysis.

Gideon Koren, MD: Study concept and design, critical revision of manuscript for intellectual content, analysis and interpretation

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Study Sponsorship

The German Multiple Sclerosis and pregnancy registry is partially funded by Biogen Idec, Bayer Healthcare, Novartis Teva Pharmaceuticals, Merck-Serono and Sanofi-Aventis.

The Motherisk Nausea and Vomiting of Pregnancy counseling line is partially funded by Duchesnay Inc.

Author Disclosure

KH: Is supported by the German Research Council (Deutsche Forschungsgemeinschaft – DFG He 6841/1-1) and has received speaker honoraria from Biogen Idec, Teva Sanofi Aventis, Novartis, Bayer Healthcare and Merck Serono.

GK: Serves as a consultant for Novartis and Duchesnay;

RG: Has received payments for consultancy from Biogen and Teva; speaker honoraria and research grants from Biogen Idec Germany, Teva, Sanofi Aventis, Novartis, Bayer Healthcare and Merck Serono.

LA: Has served as a consultant for Biogen, Acorda, Novartis, Genzyme and Questcor.

NE & SH, Declare no further conflict of interest.
Pregnancy and fetal outcomes following natalizumab exposure in pregnancy. A prospective controlled observational study

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1. ABSTRACT

Background

Safety data on first trimester natalizumab exposure are scarce, as natalizumab is usually withdrawn 3 months before pregnancy.

Objective

To investigate the fetal safety of exposure to natalizumab (Tysabri®) during the first trimester of pregnancy using disease-matched (DM) and healthy control (HC) comparison groups.

Methods

101 German women with RRMS exposed to natalizumab during the first trimester of pregnancy were identified. Birth outcomes in the exposed group were compared to a DM group (N=78) with or without exposure to other disease modifying drugs, and a HC group (N=97).
Results
A total of 77, 69 and 92 live births occurred in the Exposed, DM and HC groups respectively. The rates of major malformations ($p=0.67$), low birth weight (<2500 grams) ($p=1.0$), premature birth ($p=0.37$) did not differ between groups. Higher miscarriage rates ($p=0.002$) and lower birth weights ($p=0.001$) occurred among the Exposed and DM groups, as compared to the HC, however there was no significant difference between the Exposed and DM groups.

Conclusion
Exposure to natalizumab in early pregnancy does not appear to increase the risk of adverse pregnancy outcomes in comparison to a DM group not exposed to natalizumab.

2. INTRODUCTION
Natalizumab (Tysabri® by Biogen Idec) is a humanized monoclonal antibody indicated for the treatment of active Relapsing Remitting Multiple Sclerosis (RRMS), and Crohn’s disease. It acts as an immunomodulator by antagonizing the $\alpha4$ subunits of the $\alpha4\beta1$ and $\alpha4\beta7$ integrin molecules in the immune system; and blocking the $\alpha4$-mediated adhesion of pro-inflammatory cells to vascular endothelium and their subsequent crossing of the blood brain barriers.\textsuperscript{1,2} $\alpha4$- integrins play an active role in the fertilization, implantation, placental and cardiac development, and when antagonized with a synthetic compound in pregnant animals, severe defects
have been reported.\textsuperscript{3} Hence it is reasonable to assume risks associated with natalizumab exposures during pregnancy and a potential adverse effect on the developing fetus. In several studies in pregnant guinea pigs and cynomolgus monkeys, natalizumab exposure failed to show teratogenic effects and yielded mixed results for increased risk in abortion rates.\textsuperscript{3-5}

The registry launched by Biogen Inc, \textit{Tysabri Pregnancy Exposure Registry} (TPER), with 364 exposed pregnancies, revealed a birth defect rate of 9.5\% with no particular clustering in the pattern of defects.\textsuperscript{6} One small study with 35 accidental exposures was previously reported by Hellwig et al, with no increase in the baseline risk for defects in comparison to a group of women on other disease modifying drugs\textsuperscript{7}. Large controlled studies regarding natalizumab exposures in pregnancy are still lacking.

As many RRMS patients gain excellent control of their highly active disease with natalizumab, and studies have shown a return to baseline disease activity upon interruption of natalizumab, some as early as 4 weeks, the decision to discontinue therapy three months prior to pregnancy must be made cautiously. Many women do not conceive right away and are at high risk of experiencing relapses while off natalizumab.

The objective of the present study was to compare pregnancy outcomes in women with RRMS exposed to natalizumab to a disease-matched (DM) and healthy control (HC) groups of women.

\textbf{3. METHODS}

In 2006 a nationwide independent pregnancy registry for MS was established in Bochum, Germany. Pregnant women registered to this database were
followed prospectively either through telephone interviews every 3 months, or by visits to the university based outpatient clinic in Bochum. All self-reported data were collected through standardized questionnaires as previously described.7

For this study women accidentally exposed to natalizumab during pregnancy were recruited. Exposure was defined as treatment with natalizumab from 8 weeks prior to the start of the last menstrual period (LMP) and onward. Women were followed till 6 months postpartum. All had confirmed diagnosis of RRMS, and were recruited between 2006-2013. 35 outcomes in this group were previously reported and included in this study to increase power, and because new information was available in some of those outcomes. The Motherisk program at The Hospital for Sick Children in Toronto, Canada was established in 1985 and is a clinical, teaching and research program affiliated with the University of Toronto. It has been providing evidence-based information on drug safety in pregnancy and lactation. Two prospective Motherisk cohorts were used to serve as comparison groups for this study.

The first comparison group is a disease-matched (DM) group which consisted of pregnant women with confirmed RRMS that were recruited prospectively between 1997 and 2013, and were not treated with natalizumab. Information on maternal characteristics, gestational age at recruitment, maternal age at conception, pre-pregnancy maternal BMI, smoking and drinking status, obstetrics history, concomitant medications taken in pregnancy, and educational status were collected.
The second comparison group was the healthy control (HC) group which consisted of women with no teratogenic exposures in pregnancy. These women had contacted the Motherisk general or nausea and vomiting of pregnancy (NVP) lines between 1997-2012 to inquire about safety of non-teratogenic drugs, or ways to manage their NVP. All women were followed prospectively and had provided information on outcomes of their pregnancies. These women were matched to the Exposed group by maternal age at conception (±2 years), BMI, and gestational age at recruitment (±1 week).

The pregnancy outcomes collected included congenital malformations, spontaneous abortions (SA), therapeutic abortions (TA), gestational age (GA) at pregnancy loss, GA at birth, birth weight, head circumference, birth length, gender, and mode of delivery. Details of neonatal health and birth defects were confirmed with the child’s physician by letter communication.

**Statistical Analysis**

One way ANOVA was used to compare the means of normally distributed continuous data among the three groups. In cases where significant difference was found, post hoc analysis using Tukey’s test was conducted. For non-parametric continuous data, Kruskal Wallis test was used to compare medians. Categorical data were compared among groups in 2x3 tables using Chi Square or Fisher Exact for parametric and non-parametric data respectively. In comparisons where a significant difference was detected (P<0·05) Bonferroni’s correction was applied and the groups were
compared two at a time with the corrected p-value.

4. RESULTS

We included 276 women in total: 101 natalizumab exposed (Exposed), 78 disease-matched (DM), and 97 healthy controls (HC). Women in the DM group were significantly older compared to HC and Exposed ($p<0.001$), and the rate of alcohol exposure was significantly higher in the HC group compared to both DM and Exposed groups ($p=0.003$). Statistical analyses revealed no significant differences among the groups on GA at recruitment, pre-pregnancy BMI, smoking status, or obstetric history. Baseline characteristics of the 3 groups are presented in Table 1.

The Exposed group included 101 women with RRMS reporting on 102 pregnancy outcomes. 100 reported on singleton pregnancies and one woman a twin pregnancy. The median duration of therapy with natalizumab before pregnancy was 18·9 months. Length of exposure to natalizumab during pregnancy can be divided into four periods: 8 weeks prior to LMP ($n=20$), 0-9 weeks of gestation ($n=76$), 10-13 weeks of gestation ($n=4$). Only one woman chose to continue till the 31st week of pregnancy and her results have been previously reported. 21 women experienced a relapse during pregnancy that required at least one course of steroid treatment.

The DM group included 78 women with RRMS reporting on 95 pregnancy outcomes. Eleven women reported on two pregnancies, two women reported on three pregnancies and one twin pregnancy. Disease duration
was reported for 68 pregnancies and the median was 5.6 years at time of pregnancy. 68 women were on disease modifying drugs (DMD) prior to pregnancy: 53 (56.4%) were on interferon beta-1a/1b (IFNB), 11 (11.7%) on glatiramer acetate (GLAT), 3 (3.2%) on natalizumab, and one on fingolimod. 22 women were not receiving any DMDs prior to pregnancy. Only 25 women had gestational exposure to DMDs mostly during the first trimester and seven continued throughout pregnancy. The most common comorbidity in this group was depression and anxiety. No relapses requiring high dose steroids were reported in this group. Table 2 summarizes the differences between DM and Exposed groups.

The HC group included 97 women. Most women had called to inquire about mild to severe NVP management and had no significant medical history. The most common medical condition reported in this group were mood disorders (n=21), thyroid related conditions (n= 8), migraines (n=7) and inflammatory bowel disease (n=4). The most commonly used therapy in this group was dimenhydrinate, ginger, vitamin B6, and Diclectin® (pyridoxine & doxylamine succinate) for the management of NVP.

**Pregnancy Outcomes**

The rate of live births was significantly higher in the HC than both the Exposed and DM groups (p=0.0004). This corresponded to significantly higher rates of miscarriage in both the Exposed 17.3% and DM 21.1% groups in comparison to HC 4.1% (p=0.002) (Table.3). Nine out of twenty of the miscarriages among the DM occurred in women older than 35 years, and three of these women had two miscarriages each. Since miscarriage rates
increase after age 35, and women with one miscarriage are at a higher risk for subsequent miscarriages, a recalculation of spontaneous abortion rates in both groups after excluding women over the age of 35, changed the miscarriage rates to 11.5% and 12% in DM and Exposed groups respectively.

The mean birth weights were significantly lower in both Exposed (3159 ±478.9 grams) and DM (3198.3±515.3 grams) groups in comparison to the HC (3436.7±549.5 grams) (p=0.001). When excluding women receiving high dose steroids for relapse treatment in the Exposed (n=21), the difference in birth weights was only significant between the DM and HC groups. There was a trend toward higher birth weight percentiles in the HC babies: 59.2% versus 44.8% and 43.6% in Exposed and DM respectively, but this difference did not reach statistical significance (Table 3).

Data on birth length was available in only ten of the subjects in the HC and while a significant difference was detected (p=0.003) the paucity of data in this group precludes definite interpretation (Table 3).

There was no significant difference in the number of therapeutic abortions, gestational age at birth, gender, head circumference, premature births (<37 weeks), low birth weights (<2500 grams) and birth defects among the three groups (Tables 3&4).
**Delivery Outcomes**

The rate of vaginal delivery was significantly higher in HC compared to the Exposed ($p=0.006$) but did not differ from the DM. The rate of scheduled cesarean-sections (SCS) was significantly higher in the Exposed ($^{23}_{73}$; 31.5%) compared to both DM ($^{4}_{66}$; 6.1%) and HC ($^{5}_{62}$; 8.1%) ($p<0.0001$). The rate of emergency cesarean-sections (ECS) were significantly higher in DM in comparison to the Exposed ($p=0.02$) but did not differ from HC group. Table 4 summarizes the delivery outcomes for the three groups.

**Birth Defects**

Birth defects were detected in ($^{3}_{77}$; 3.9%), ($^{1}_{69}$; 1.4%), and ($^{2}_{92}$; 2.2%), of the live births in the Exposed, DM and HC groups respectively with no significant difference among the groups ($p=0.67$) (Table 4).

In the *Exposed Group*, there was an atrial septal defect (ASD) in a fullterm female baby; hernia in a premature male; and one term male baby with hexadactyly. All three mothers had discontinued natalizumab in the first week of conception. In addition, one fullterm male baby was diagnosed with neuroblastoma, hepatomegaly, renal and hepatic insufficiency, sepsis and developmental retardation shortly after birth.

Amongst the non-live births three fetuses with genetic anomalies were reported —one case of Trisomy 16, one case of Heterotaxy syndrome with complete atroioventricular septal defect (AVSD) and defected azygos, and
one case of Turner Syndrome Mosaicism. All three women had
discontinued natalizumab within the first few weeks of pregnancy (Table 5).

In the DM group, there was one case of clubfoot in a premature female baby
requiring three surgeries. The mother had reported that clubfoot runs in the
family. She had used GLAT all through her pregnancy. Additionally one
case of cryptorchidism, one case of double hernia, and one hypospadias were
also reported. These three cases were not included in the count of major birth
defects as they were medically unconfirmed and their data were insufficient
(Table 5).

In the HC group, there was one case of ureter pelvic junction (UPJ)
obstruction in a premature baby, and one case of ventricular septal defect
(VSD) in a premature male.

**Retrospectively Exposed Cases**

Four women in the Exposed group were recruited retrospectively, after
pregnancy outcomes were confirmed. The mean disease duration was 7.4
years and one of the four women smoked during pregnancy (10
cigarettes/day). All four women gave birth to fullterm babies; three
vaginally and one by emergency cesarean section. There were three males
and one female. The mean birth weight was 3251±376 grams, and no birth
defects or adverse outcomes were reported in this group.
5. **DISCUSSION**

This is the largest study to date reporting on fetal outcomes following natalizumab exposure during the first trimester of pregnancy using diseased matched and healthy comparison groups. A major strength of this study is the use of two comparison groups with HC women closely matched to the Exposed women on maternal age at conception, BMI and gestational age at recruitment, all of which are well known confounders of pregnancy outcomes. Critically, having a DM group to account for the effects of MS itself on pregnancy outcomes, allows the identification of any potential impact that natalizumab may have exerted.

Our findings in this study demonstrated no apparent increase defects rates in live births of the Exposed group compared to both DM and HC. This result is biologically plausible, as immunoglobulins begin to cross the placenta more readily after 22 weeks of gestation, long after embryogenesis has been completed.\(^1^0\) Furthermore we did not observe an increase in risk for other adverse neonatal outcomes such as low birth weight (< 2500grams) prematurity and reduced head circumference in the Exposed as compared to the DM and HC group babies.

The observed birth weights and birth lengths while similar between the Exposed and DM groups were significantly lower than the HC group. Similarly while the percentile birth weights did not differ significantly between the three groups, they were lower in the two MS groups. Lower birth weight and birth length among babies born to mothers with MS have
been previously reported.\textsuperscript{11,12} A neurologically based defect in pelvic circulation may be potentially contributing to this observation.\textsuperscript{11} The observed incidence rates of spontaneous abortions while higher in the Exposed and DM groups was only significantly different from the HC group. However several selection biases may have impacted this result. First, the majority of HC women had sought counseling on ways to manage their nausea and vomiting of pregnancy (NVP), and severity of (NVP) is associated with a decreased risk for spontaneous abortions\textsuperscript{13}. Thus the observed miscarriage rates in the HC group may be lower than the general population rate, leading to a larger difference between HC rates and the rates in the two MS groups.

Secondly, women in the DM group were recruited 2.7 weeks earlier; had higher maternal age at pregnancy, and some had exposure to DMDs in pregnancy. While the difference in gestational age at recruitment was not statistically significant, and almost identical miscarriage rates were seen in DM and Exposed groups after adjusting for maternal age, and despite a 2012 review claiming there is no strong evidence for increased risk in spontaneous abortions with DMD exposures,\textsuperscript{14} we cannot conclude that the higher miscarriage rates in the DM and Exposed is due to the disease alone. Further investigation using a DM group closely matched on criteria such as disease severity, expanded disability status scores (EDSS) scores, relapses and steroid use during pregnancy, DMD usage and obstetric history, would isolate the effect of natalizumab on spontaneous abortion rates more precisely.
The observed rate of vaginal delivery was lowest in the Exposed group which was significant in comparison to the HC group only. Correspondingly we observed a much higher rate of scheduled C-sections in the Exposed compared to both comparison groups. The difference in mode of deliveries might be due to differences between the two countries. In Germany the nationwide cesarean section rate is about 30% and therefore comparable to the natalizumab exposed pregnancies. The severity of the disease itself may also pose as an impediment to normal vaginal deliveries in women on natalizumab who are likely to have more active disease; however we are not able to confirm this as EDSS scores were not available for these women due to the nationwide collection of data and different practice amongst neurologist in performing EDSS assessments.

Another limitation of this study is the comparison of women from different countries. The Canadian comparison groups were selected, due to the availability of data on both healthy and disease matched groups with the opportunity to closely match for relevant variables known to impact pregnancy outcome such as age, BMI and gestational week of contact. It may be argued that this type of comparison may be a source of bias, mainly through differences in ethnic makeup, neonatal characteristics and obstetric outcomes.

As Canada and the United States have one of the highest immigrant population, the ethnic diversity can introduce multiple biases. However, an analysis of the ethnic makeup in our Canadian Motherisk groups, revealed a significant number of European Caucasians in both HC
(96%) and DM (93%) groups.

To address neonatal differences between countries, an analysis of national birth weights by gender in both Canada and Germany revealed exceptionally similar results with a median 3631g, 3613 g for German and Canadian males, and 3479g, 3470 g for German and Canadian females respectively at 40 weeks of gestation.\textsuperscript{15,16} Given this data, the birth weights observed, are likely to reflect the influence of the disease only.

Addressing differences in obstetric outcomes between the two countries is a complex issue as nearly 45% of pregnancies are unplanned (42% in Western Europe and 48% in North America)\textsuperscript{17} and early losses are often not recognized or undocumented even if known. Miscarriage rates in industrialized countries for known pregnancies has been estimated to be anywhere from 3% to 33%.\textsuperscript{18} A report by Statistics Canada claimed a 5% miscarriage rate in Canada from hospitalized cases.\textsuperscript{18} A study in 2010 calculated miscarriage rates in 2008 for different regions across the world and estimated a 5% and 7% miscarriage rate for Western Europe and North America respectively.\textsuperscript{17} Between 1995 and 2008 a 20% reduction in accidental pregnancies was observed worldwide with Western and Southern Europe having one of the lowest unintended pregnancy rates. In contrast, North America has shown no decline in the rates of unplanned pregnancies.\textsuperscript{17} Furthermore, North American women are much less likely to undergo induced abortions for unintended pregnancies compared to women from other countries.\textsuperscript{17} Hence, while the proportions of unintended pregnancies leading to live births may differ between Western Europe and
North America, the spontaneous miscarriage rates are not expected to vary substantially.

Finally, while a significantly higher alcohol exposure among the Canadian HC women was observed, we believe that this difference mostly reflects a few drinks that were consumed prior to mothers realizing their pregnancies and does not indicate regular consumption throughout pregnancy. Typically, women with chronic medical conditions such as MS are likely to be more health conscience and to avoid unhealthy habits, and it is not surprising that MS patients abstained from alcohol altogether.

We do acknowledge the potential for selection bias in this study. Both German and Canadian patients contacted the respective registries themselves voluntarily potentially impacting the generalizability of the findings. However for the natalizumab exposed women we believe to have captured most of the exposed pregnancies in Germany as the registry is well known and supported by neurologists, MS nurses and the national MS society. Despite the matching of women on criterions of age, gestational age at call and BMI, and the European–Caucasian roots in the Canadian women and the shared similarities in obstetric outcomes and healthcare systems between Germany and Canada, minor differences between Germany and Canada cannot be excluded.

This study allowed us to investigate the impact of natalizumab exposure on 101 pregnancies. Since the majority of the women in the Exposed group (75%) discontinued natalizumab prior to 10 weeks, our findings and
interpretations are limited to early exposure in pregnancy only. As maternal antibodies transfer minimally in the first trimester and only 5-10% between 17-22 weeks of gestation, we can surmise the same for natalizumab transfer, and that the highest fetal exposure occurs in late pregnancy, which may theoretically have an impact on neonatal immunity. A recent study demonstrated hematological abnormalities in babies exposed to natalizumab in later pregnancy.

6. CONCLUSION

Using a disease matched group, this study reveals that natalizumab does not appear to increase the baseline risk for malformations, preterm births and low birth weight babies. The risk for miscarriage however, while very similar to the DM group, may still be of concern and requires further investigation in future studies. The decision to discontinue natalizumab three months prior to planning a pregnancy, should be decided on a case by case basis, and women with exposure in early pregnancy should be reassured that termination may not be necessary.
ACKNOWLEDGEMENT

We sincerely thank Seyedeh Bahar Hashemi (BSc) for helping with data quality screening and analysis.

We sincerely thank all women and their healthcare providers who participated in this study, without whom this study would not have been possible.

AUTHOR DISCLOSURE

The German MS and pregnancy registry is partly supported by Bayer Healthcare, Biogen Idec Germany, Teva, Sanofi Aventis, Genzyme, Novartis, and Merck Serono. **KH:** Is supported by the German Research Council (Deutsche Forschungsgemeinschaft – DFG He 6841/1-1) and has received speaker honoraria from Biogen Idec, Teva Sanofi Aventis, Novartis, Bayer Healthcare and Merck Serono. **RG:** Has received payments for consultancy from Biogen and Teva; speaker honoraria and research grants from Biogen Idec Germany, Teva, Sanofi Aventis, Novartis, Bayer Healthcare and Merck Serono. **LA:** Has served as a consultant for Biogen, Acorda, Novartis, Genzyme and Questcor. **GK:** Serves as a consultant for Novartis. **NE & SH:** Declare no further conflicts of interest.

ACRONYMS

**MS:** Multiple Sclerosis

**RRMS:** Relapsing Remitting Multiple Sclerosis  **EDSS:** Expanded Disability Status Scale  **DMD:** Disease Modifying Drugs  **NVP:** Nausea and Vomiting of Pregnancy  **SA:** Spontaneous Abortion/ Miscarriage  **TA:** Therapeutic Abortion  **GA:** Gestational Age  **LMP:** Last Menstrual Period  **BMI:** Body Mass index  **ASD:** Atrial Septal Defect  **VSD:** Ventricular Septal Defect  **UPJ:** Ureter Pelvic Junction  **SCS:** Scheduled Cesarean Section  **ECS:** Emergency Cesarean Section  **GLAT:** Glatiramer Acetate (Copaxone®)  **INF-B-1a:** Interferon Beta 1a (Avonex, Rebif®)  **INF-B-1b:** Interferon Beta 1b (Beta(s/f)eron®)

TERMINOLOGY AND DEFINITIONS

**Healthy Controls (HC):** A comparison group of women with no MS and no exposure to any teratogens.

**Disease-Matched (DM):** A diseased match comparison group of women with RRMS with or without exposures to DMDs

**Exposed:** The study group of women with RRMS with accidental exposure to natalizumab in early pregnancy.
7. REFERENCES


Table 1. Maternal characteristics in each of the study groups

<table>
<thead>
<tr>
<th>Maternal Characteristics</th>
<th>Natalizumab Exposed</th>
<th>Disease Matched</th>
<th>Healthy Controls</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age (yrs)</td>
<td>30·5±5·3‡</td>
<td>33·9±4·7‡</td>
<td>30·6 ± 4·9∞</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>(Median)</td>
<td>(30·2)</td>
<td>(33·3)</td>
<td>(30·5)</td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±Sd (Median)</td>
<td>N=61</td>
<td>N=56</td>
<td>N=69</td>
<td>0·47</td>
</tr>
<tr>
<td>24·4±5·3 (23·8)</td>
<td>24·9±4·6 (24·2)</td>
<td>23·8±4·5 (23·3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at call</td>
<td></td>
<td></td>
<td></td>
<td>0·07</td>
</tr>
<tr>
<td>(Weeks)</td>
<td>N=97</td>
<td>N=79</td>
<td>N=97</td>
<td></td>
</tr>
<tr>
<td>Mean±Sd (Median)</td>
<td>12·5±9·5 (6·0)</td>
<td>9·4±9·5 (6·0)</td>
<td>12·2±9·1 (8·9)</td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>1·0 (1·0-2·0)</td>
<td>1·0 (1·0-2·0)</td>
<td>2·0 (1·0-3·0)</td>
<td>1.0^</td>
</tr>
<tr>
<td>Parity</td>
<td>0·0 (0·0-1·0)</td>
<td>0·0 (0·0-1·0)</td>
<td>1·0 (0·0-1·0)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>0·0 (0·0-0·0)</td>
<td>0·0 (0·0-0·0)</td>
<td>0·0 (0·0-0·0)</td>
<td></td>
</tr>
<tr>
<td>Terminations</td>
<td>0·0 (0·0-0·0)</td>
<td>0·0 (0·0-0·0)</td>
<td>0·0 (0·0-0·0)</td>
<td></td>
</tr>
<tr>
<td>Alcohol Exposure</td>
<td>1/90 (1·1%)</td>
<td>4/94 (4·3%)</td>
<td>11/86 (12·8%) ∞</td>
<td>0·003*</td>
</tr>
<tr>
<td>Smoking Exposure</td>
<td>8/86 (9·3%)</td>
<td>8/94 (8·5%)</td>
<td>10/85 (11·8%)</td>
<td>0·75</td>
</tr>
</tbody>
</table>

Table 2. Disease details of patients with multiple sclerosis in the Exposed and Disease-Matched groups

<table>
<thead>
<tr>
<th>Disease Characteristics</th>
<th>Natalizumab Exposed</th>
<th>Disease Matched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (N total)</td>
<td>101</td>
<td>78</td>
</tr>
<tr>
<td>Total Outcomes</td>
<td>102</td>
<td>95</td>
</tr>
<tr>
<td>Prospective Outcomes</td>
<td>98/102 (95%)</td>
<td>84/95 (88%)</td>
</tr>
<tr>
<td>Disease Duration (Median years)</td>
<td>5.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Age at MS Diagnosis (Mean±Sd years)</td>
<td>23.7±5.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Exposed to DMDs in Pregnancy N(%)</td>
<td>101 (100%)</td>
<td>25 (32%)</td>
</tr>
<tr>
<td>Type of DMD exposure (N)</td>
<td>Natalizumab (101)</td>
<td>Glatiramer Acetate…..(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INF1b.............(6)</td>
</tr>
</tbody>
</table>

^Kruskal Wallis Test; *Fisher Exact; ±/‡ matching pairs differ significantly as identified by Tukey’s test
Table 3. Pregnancy outcomes in cohorts

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Exposed</th>
<th>Disease Matched</th>
<th>Healthy Controls</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Births</td>
<td>77/98 (78.6%)</td>
<td>69/95 (72.6%)</td>
<td>92/98 (93.9%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Termintations</td>
<td>4/98 (4-1%)</td>
<td>6/95 (6-3%)</td>
<td>2/98 (2-0%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>17/98 (17-3%)</td>
<td>20/95(21-1%)</td>
<td>4/98 (4-1%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Gender</td>
<td>M: 38/76 (50%)</td>
<td>M: 17/49 (32%)</td>
<td>M: 18/36 (50%)</td>
<td>0-20</td>
</tr>
<tr>
<td>F: 38/76 (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>38-8±1-6 (38-9)</td>
<td>38-5±2-0 (38-6)</td>
<td>39±1-7 (39-8)</td>
<td>0-17</td>
</tr>
<tr>
<td>(weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Weight (grams)</td>
<td>3159±478-9</td>
<td>3198-3±515-3</td>
<td>3436-7±549-5</td>
<td>0-001</td>
</tr>
<tr>
<td>Birth Weight (No Steroids)</td>
<td>3247-6±396-4</td>
<td>3198-3±515-3</td>
<td>3436-7±549-5</td>
<td>0-01*</td>
</tr>
<tr>
<td>Median (3255)</td>
<td></td>
<td>Median (3247-5)</td>
<td>Median (3444-5)</td>
<td></td>
</tr>
<tr>
<td>Birth Weight %</td>
<td>44-8</td>
<td>43-6</td>
<td>59-2</td>
<td>0-13^</td>
</tr>
<tr>
<td>Median (25%-75%)</td>
<td>(25-1-70-2)</td>
<td>(23-66-5)</td>
<td>(22-9-90-0)</td>
<td></td>
</tr>
<tr>
<td>Head Circumference Mean±Sd(cm)</td>
<td>35-1±2-2</td>
<td>34-2±1-9</td>
<td>34-1±2-2</td>
<td>0-30</td>
</tr>
<tr>
<td>Birth Length Mean±Sd(cm)</td>
<td>50-3±2-5</td>
<td>50-6±3-4</td>
<td>53-5±2-1</td>
<td>0-003</td>
</tr>
</tbody>
</table>

*Excluding s of babies exposed to steroids (N=21) during relapse treatment(s) in natalizumab exposed pregnancies;  
^ Kruskal Wallis Test  
∞/‡ Matching pairs differ significantly as per Tukey’s test
**Table 4.** Adverse neonatal outcomes and mode of delivery

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Natalizumab Exposed</th>
<th>Disease-Matched</th>
<th>Healthy Controls</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Anomalies</td>
<td>4/77 (5.2%)</td>
<td>3/69 (4.3%)</td>
<td>5/92 (5.4%)</td>
<td>1.0**</td>
</tr>
<tr>
<td>Major Birth Defects</td>
<td>3/77 (3.9%)</td>
<td>1/69 (1.4%)</td>
<td>2/92 (2.2%)</td>
<td>0.67**</td>
</tr>
<tr>
<td>Premature (&lt;37wks)</td>
<td>6/76 (7.9%)</td>
<td>10/67 (14.9%)</td>
<td>9.0/92 (9.8%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500g)</td>
<td>6/77 (7.8%)</td>
<td>5/68 (7.4%)</td>
<td>7.0/92 (7.6%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Delivery Method**

<table>
<thead>
<tr>
<th></th>
<th>Natalizumab Exposed</th>
<th>Disease-Matched</th>
<th>Healthy Controls</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td>36/73 (49.3%)</td>
<td>43/66 (65.2%)</td>
<td>47/62 (75.8%)</td>
<td>0.0006</td>
</tr>
<tr>
<td>C/S- Planned</td>
<td>23/73 (31.5%)</td>
<td>4/66 (6.1%)</td>
<td>5/62 (8.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C/S –Emergency/Repeat</td>
<td>8/73 (11.0%)</td>
<td>19/66 (28.7%)</td>
<td>10/62 (16.1%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Kruskal Wallis Test; Fisher Exact; matching pairs differ significantly as identified by Tukey’s test*
CHAPTER 3
MANUSCRIPT
TWO

GESTATIONAL VITAMIN D AND POSTPARTUM DISEASE

Vitamin D Supplementation in Multiple Sclerosis: A Comparative Study of Two Cohorts

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Funding
The German MS and Pregnancy Registry is partly supported by Biogen Idec, Bayer, Novartis Teva, Merck, and Sanofi.
The Motherisk MS Pregnancy Registry was funded by Novartis
Authors Contribution

Neda Ebrahimi, M.Sc.: Study concept and design, data acquisition, analysis, interpretation, drafting and revision of manuscript.

Sandra Herbstritt M.Sc.: study design, data acquisition, and analysis.

Ann Yeh MD: Study concept and design, critical revision of manuscript for intellectual content, analysis and interpretation

Richard Gladstone MD: Study concept and design, critical revision of manuscript for intellectual content, analysis and interpretation

Manny Papadimitropoulos PhD: Study concept and design, critical review of intellectual content, analysis and interpretation.

Shinya Ito MD: Study concept and design, critical revision of manuscript for intellectual content, analysis and interpretation, study supervision

Kerstin Hellwig MD: Study concept and design, data acquisition, critical revision of manuscript for intellectual content, analysis and interpretation and study supervision

Keywords

Vitamin D, Calcidiol, Pregnancy, Gestational, Multiple Sclerosis, Relapsing Remitting Multiple Sclerosis, MS, RRMS, Women, Supplementation, Vitamin D intake, Postpartum relapse
I. ABSTRACT

OBJECTIVE
To evaluate the effect of gestational supplementation of Vitamin D on postpartum disease course of Relapsing Remitting Multiple Sclerosis (MS) by comparing two cohorts—a Vitamin D supplementing Canadian cohort and a non-supplementing German cohort.

METHODS
A retrospective analysis of two Pregnancy MS registries (German (GER) and Canadian (CAN)) was conducted. Both registries collected similar data on maternal characteristics, obstetrics, disease history, disease course, exposures throughout pregnancy, exposure in the twelve months postpartum, and dosage of Vitamin D (in the Canadian Cohort). The primary outcome of interest, time to first postpartum relapse, was determined by using the Kaplan-Meier method. Adjusted and unadjusted hazard ratios (HRs) were calculated using cox proportional hazard regression methods. The adjusted model corrected for maternal age, pregnancy relapses and duration of breastfeeding.

RESULTS
A total of 191 women were registered in to the cohorts (82 in CAN and 109 in GER). The median dose of Vitamin D was 2422 (IQR: 1043-4250) IU/ day in the CAN. There were no major differences between cohorts on maternal characteristics, and disease severity. Usage of disease modifying drugs (DMD) during pregnancy and postpartum was significantly higher, and breastfeeding duration significantly shorter in the GER group. Although the GER group showed a significantly longer time to
first postpartum relapse, this difference was no longer significant after adjusting for maternal age, the number of relapses during pregnancy, and duration of breastfeeding. There was no significant difference in the total number of postpartum relapses.

CONCLUSION

Despite the greater usage of DMDs in the GER group, there was no significant difference in postpartum MS resurgence between the cohorts. Vitamin D supplementation may offer some beneficial effects on MS disease course in the postpartum period. Further studies are required to confirm this finding.
2. **INTRODUCTION**

Vitamin D is a secosteroid hormone produced endogenously through ultraviolet B irradiation of the skin and through exogenous intake of foods high in Vitamin D such as fatty fish, or through supplementation. In the body, Vitamin D is converted into 25-hydroxyVitamin D (25(OH)D/calcidiol), which can be further metabolized by the kidney to several active metabolites; the most studied of which is 1,25-dihydroxyVitamin D (1,25(OH)₂D). ¹ Many organs and tissues, including immune cells, are able to convert calcidiol to its active metabolite and to utilize it in an autocrine fashion. ¹ The immunomodulatory effect of Vitamin D has been long known, and its deficiency is a recognized environmental risk factor for the onset of multiple sclerosis (MS). ² More recently some studies have linked Vitamin D to MS disease activity³⁻⁵ and clinical outcomes⁶⁻⁸. The most common indicator of Vitamin D status is serum calcidiol; many studies examining the role of serum calcidiol levels in patients use this maker to determine deficiency (<50nmol/L), insufficiency (50-74nmol/L) and sufficiency (≥75nmol/L). ¹ While no consensus has been reached, there is evidence that what is currently classified as sufficient serum levels may not be adequate. ⁹ Several studies suggest that ≥100 nmol/L may be required to observe favorable immunomodulatory effects. ⁹,⁵ Because Vitamin D status is influenced by many factors including genetics, body composition, skin color, gender, sunlight exposure, age, pregnancy and breastfeeding, it is challenging to
establish optimal serum levels and its corresponding dose in the majority of population. During pregnancy the fetus’ demand for Vitamin D is met by active transfer of maternal calcidiol through the placenta which contains its own hydroxylase enzyme capable of converting maternal calcidiol to the active form. In late pregnancy, particularly the last month, the increasing demand of transplacental calcium transfer is met by the increase in metabolism of maternal calcidiol to its active form resulting in a progressive increase in total maternal serum $1,25(OH)_2D$ levels. The depletion of calcidiol stores during pregnancy can be further aggravated with breastfeeding. During breastfeeding the calcium and Vitamin D needs of the newborn lead to an increase in maternal calcium absorption via higher metabolism of calcidiol to $1,25(OH)_2D$ and through the breastmilk transfer of maternal pre-hormone Vitamin D.

Hence insufficiency (serum calcidiol levels <75nmol/L) commonly occurs in healthy pregnant and breastfeeding women. Interestingly Jelkanen et al. reported maternal serum calcidiol levels to be significantly lower in women with MS compared to controls both during pregnancy and postpartum.

It is well established that in the postpartum period, particularly in the first 3-6 months, there is heightened MS disease activity, typically more intense than the pre-pregnancy year. The higher risk of postpartum relapses in the MS cohort, along with declining serum calcidiol levels especially in breastfeeding mothers, and the link between low Vitamin D serum levels and MS relapses, necessitates the evaluation of the efficacy of supplementation with Vitamin D for these women. In this study we
examined the impact of supplementation with Vitamin D on postpartum MS disease course, by comparing a group of women that have consistently supplemented with Vitamin D for several years prior to conception and throughout pregnancy to a group of women that have not been supplementing with Vitamin D during pregnancy. Our objective was to elucidate the impact of Vitamin D supplementation on postpartum disease course in women with relapsing remitting MS by comparing postpartum relapses in two cohorts from two different MS pregnancy registries: one in Canada with Vitamin D supplementation and the other in Germany without Vitamin D supplementation.

3. METHODS

Study Cohort

Each of the two cohorts belonged to a Pregnancy and MS Registry: one established in Bochum, Ruhr Germany; and the other in Toronto, Canada. These two cohorts were distinct in their pregnancy Vitamin D supplementation with regular prenatal and Vitamin D supplementation in the Canadian patients and none in the German cohort.

*German Cohort (GER):* The nationwide German MS and Pregnancy Registry was established in 2008 at Ruhr University in Bochum, Germany. Enrolled pregnant women with MS were interviewed every three months using structured questionnaires about the course of pregnancy, disease, relapses and exposures. Enrollment of these women was mainly through self-referral, and the details of this registry were previously published.15
Women who were taking Vitamin D or prenatal vitamins were excluded from the German cohort.

*Canadian Cohort (CAN):* The Canadian MS registry was established at the Hospital for Sick Children in 2011 as part of the multicenter international study: “Gilenya® (fingolimod) Exposure in Pregnancy”, funded by Novartis, and enrolled pregnant women with relapsing remitting MS not exposed to fingolimod. Upon enrollment, patients completed four phone interviews: the first interview was the baseline interview at enrollment, second during pregnancy, third after delivery/miscarriage, and fourth twelve months after delivery. Detailed information on maternal health status, neonatal health, number of relapses and treatments was collected at each interview.

**Data Acquisition**

Usage of data from both of these registries was approved by the research ethics board of the corresponding home institution: Ruhr University in Bochum Germany, and SickKids Hospital in Toronto, Canada. For the purpose of the present study, anonymized registry data was combined into a single dataset. Extracted data included the following: maternal characteristics (age, BMI, gestational age at enrollment, LMP, educational status, obstetric history); MS history (age at diagnosis, disease duration, treatment, number of relapses within 1.5 years prior to pregnancy, during and post pregnancy); drug and vitamin exposures (start/stop dates, dosage and start/stop dates of Vitamin D in the Canadian cohort); and pregnancy,
delivery and neonatal outcomes.

**Relapse Definition**

Relapses in both cohorts were defined as the occurrence of new and/or recurrence or exacerbation of old MS neurological symptoms lasting more than 24 hours in the absence of fever, infections or other causes that may have explained these symptoms. Relapses were examined after occurrence and confirmed by a neurologist only in the GER. In the CAN the symptoms and duration of each relapse were self-reported at the time of each interview. Prior to analyzing the data, three neurologists (KH, AY, RG) independently reviewed the details of each episode using the standard definition above, and confirmed whether the event could be classified as a relapse or not. Events that were confirmed by at least two neurologists to be a relapse were counted as true relapses in these patients.

**Statistical Analysis**

Normally distributed continuous data was compared between the two cohorts using independent sample t-test, or Mann Whitney U-test for non-parametric data. Categorical data was compared using Chi Square or Fisher Exact. The primary outcome, time to first postpartum relapse, was compared between the two groups using Kaplan Meier Survival method. In order to account for censoring (women that didn’t experience a relapse or were lost to follow-up), a 25 month follow-up cut off time was used and women that did not experience a relapse by 25 months were censored.

To account for the effect of confounders we performed a logistic regression analysis to identify predictors of relapse risk. Factors known to impact
maternal serum calcidiol levels (i.e., maternal BMI, age at conception, number of weeks of pregnancy spent in fall or winter seasons [October 1st-March 31st], breastfeeding (yes/no), breastfeeding duration) and factors that have been reported in the literature to impact postpartum disease activity (i.e., disease duration, frequency of relapses prior to and during pregnancy, duration of exclusive breastfeeding, usage of DMDs in pregnancy or postpartum) were included and assessed in the regression model.

Adjusted and unadjusted hazard ratios were calculated using cox proportional hazards regression methods for the entire cohort. Each confounder identified by the regression analysis explained above was included in the cox proportional hazard model to calculate adjusted hazard ratios for the entire cohort.

For exploratory purposes we repeated the analysis for several subgroups to remove the potential effect of confounders that were not identified as significant predictors of relapse risk in our logistic regression. We calculated adjusted and unadjusted hazard ratios for the following subgroups:

- Women with pre-pregnancy BMI less than 25
- Women with twenty or more weeks of their pregnancies spent in the winter/fall months
• Women with pre-pregnancy BMI< 25 and who spent less than 20 weeks of their pregnancy in the fall/winter months
• Women with no relapses during pregnancy
• Women with early DMD initiation (≤2 months of delivery)
• Women with delayed DMD initiation (≥6 months or later after delivery)
• Women that exclusively breastfed for at least 2 months
• Women that breastfed while taking DMDs

5. RESULTS

A total of 191 women were enrolled in both registries: 82 women in the CAN and 109 women in the GER. A total of 89 pregnancies were registered in CAN (7 of 82 women registered two separate pregnancies); there were 70 live births (1 set of twins), 6 miscarriages and 14 were lost to follow-up. In GER, there were 103 live births and 6 miscarriages, and none were lost to follow-up.

There were no significant differences in pre-pregnancy weight, pre-pregnancy BMI, age at conception, gestational age at enrollment nor in the rates of past miscarriages and terminations between groups (Table 1). Significant differences were observed between the groups in height, obstetric history (gravida and parity), highest level of attained education, and duration of postpartum follow-up as explained below.

GER had more pregnancies and more children. By the end of the follow-up
period, 51.8% of CAN and 2.8% of GERs had one pregnancy in total, and 26.5% of CAN and 64.2% of GERs had at least two children. The CAN group was significantly more educated than the GER group. Duration of postpartum follow-up in the GER group was longer than the CAN group: 14.6±7.9 vs. 11.8±6.5 months, respectively.

There was no significant difference between the groups on the age of MS diagnosis, or the duration of disease at conception (Table 2). However, more women in the GER cohort (85.3%) were exposed to disease modifying drugs (DMD) during pregnancy than the CAN cohort (22.2%). Likewise, significantly more women in the GER cohort initiated DMDs postpartum (98.1%) than CAN (50.0%). Overall the duration of DMD interruption was significantly shorter in GER (10.6 months) than CAN (25.7 months).

Table 3 compares breastfeeding behavior between the cohorts. Significantly more women in CAN breastfed their babies, and did so for a longer duration of time. Similarly, the duration of exclusive breastfeeding was significantly longer in the CAN cohort. Lactational amenorrhea (a surrogate marker of breastfeeding duration and intensity) was longer in the Canadian cohort as expected.

Our main interest was postpartum relapses (Table 5). In the postpartum period significantly more women in GER (58%) were relapse free than those in CAN (41%). In the 18-month prior to pregnancy and during pregnancy, percentages of relapse-free women did not differ significantly
between the cohorts (Table 4).

In women that did experience at least one postpartum relapse, the median time to the first relapse was 3.5 and 2.7 months postpartum in CAN and GER groups respectively. After accounting for censoring, the Kaplan Meier analysis indicated a significantly higher risk of postpartum relapse in the CAN. The mean time to first postpartum relapse was 13.41 (95%CI: 10.99-15.84) months in CAN and 16.96 (95%CI: 15.02-18.90) months in GER (Figure 1).

In the logistic regression analyses, maternal age at conception, number of relapses during pregnancy, and breastfeeding duration were shown to independently predict postpartum relapse risk; higher maternal age at conception was associated with a lower risk while longer duration of breastfeeding and higher number of pregnancy relapses were associated with a higher risk ($X^2(3)=19.05$, $p=<0.001$). With these covariates, the adjusted hazard ratios did not differ significantly between the groups (Fig 2; Hazard Ratio 1.11; 95% CI (0.69 – 1.78), $p=0.67$).
Table 6 summarizes results of the cox proportional hazard analysis.

Adjusted and unadjusted hazard ratios for the entire cohort and for specific subgroups are provided. In the unadjusted model we observed a 57% increased risk of postpartum relapse in the whole CAN cohort (p=0.036); after adjusting, the risk was only 11% higher and was no longer significant (p=0.673). The unadjusted risk was also significantly higher in CAN when stratifying women with long winter/fall pregnancies (114% higher), women with no relapses during pregnancy (73% higher) and women with delayed initiation of postpartum DMDs (169% higher). However, after adjustment the differences were no longer significant.

Interestingly in the unadjusted and adjusted models, the risk in the CAN cohort appeared to lower by 22% and 31% respectively when analyzing
women with normal BMIs who spent less than 19 weeks of their pregnancies in the fall/winter months although this was not statistically significant (p=0.507 & 0.328 respectively). The difference between cohorts was also not significant in subgroups of women that started DMDs within two months of delivery, nor in women that exclusively breastfed for at least two months. Details of the subgroup analysis are included in the supplementary section.

6. DISCUSSION

This study characterizes postpartum disease course in pregnant women with Relapsing Remitting MS in the context of regular Vitamin D supplementation. To this end, we compared a Canadian cohort that supplemented with Vitamin D to a disease matched German group that did not. Our results suggest no significant difference between groups in timing of disease resurgence in the postpartum period. We also found no significant difference in the frequency of postpartum relapses. Furthermore by restricting analysis to specific subgroups (details of each in the supplementary section) a reduction in risk, albeit not significant, was observed in the CANs.

Presumably, healthy weight German and Canadian women conceiving in late winter (fewest gestational weeks spent in fall/winter) are less likely to have deficient calcidiol levels during pregnancy than higher BMI women conceiving in the fall. With the additional supplementation in the CAN cohort we may not only have removed confounding due to deficiency but
may have assessed risk in women with “optimal” (>100nmol/L) serum calcidiol levels. This may explain the non-significant reduction in risk when we restricted analysis to women with normal BMIs and shorter fall/winter pregnancies.

Another reduction in risk was observed in the CANs when DMDs were initiated within 2 months of delivery. Most of these were first line DMDs. Increased therapeutic benefit of DMDs in the presence of higher Vitamin D levels has been reported, and it may be that the CANs with early initiation may have experienced a more optimal response to DMDs. It is pertinent to note that the numbers are small in these subcategories and the reduction in risk was not significant. Hence we are unable to make a definite statement about the benefits of Vitamin D as an add-on therapeutic agent.

The lack of a significant difference between the two cohorts and subgroups in spite of the greater usage of DMDs in the GER is an unexpected finding. In our logistic regression model, the only variables that did significantly predict higher postpartum relapse risk were increased number of relapses during pregnancy, lower maternal age at conception and longer duration of breastfeeding. A similar relationship between postpartum disease activity and pregnancy relapses and maternal age at conception has been reported. The impact of breastfeeding on postpartum disease course has been studied, and favorable results have been reported with exclusive breastfeeding. Our findings contradict these studies and suggest a higher risk for relapse with longer breastfeeding duration and an increased risk in both groups (significant only in CAN) when we restricted analysis to women who...
exclusively breastfed for at least 2 months (table 6). However unlike the
study by Hellwig, 20 we did not have data on women’s intention on
breastfeeding prior to delivery, reasons for stopping exclusive breastfeeding
[MS related reasons (i.e. having a relapse or starting DMDs) or non-MS
related reasons (going back to work, not having enough milk, etc.). We also
were not able to compare disease severity between exclusive and non-
exclusive breastfeeders. All of these may have confounded our results and
led to the observed discrepancy from previously reported findings. If
however a true negative effect exists for breastfeeding, some potential
explanations may be the stress associated with the demanding nature of
breastfeeding, lack of sleep, longer treatment interruption and even lower
serum calcidiol levels in breastfeeding mothers. The debate between experts
on the protective effect of exclusive breastfeeding is ongoing and only
future studies will be able to address this. 22-24

The major limitation of this study is the observational design comparing two
g�ographically distinct cohorts. The environmental factors including
medical practice, diet and lifestyle may have contributed to some of the
observed difference in the two cohorts (usage of DMDs, rates of
breastfeeding, etc.) Additionally, as the cohorts belonged to registries that
were designed for a different purpose (i.e., pregnancy and fetal outcomes
following DMD exposures), some crucial data such as serum calcidiol
levels, and clinical disease severity measure were not collected; also inter
and intra-registry difference in patient referral, follow-up duration, and data
collection methods may have deepened the selection bias, making the two
cohorts less comparable. We will discuss some of the potential concerns below. The greater exposure to DMDs in the German cohort may be interpreted as more severe disease. However, we cannot exclude a possibility of differences in clinical practice between the two countries. Furthermore, many women in the Canadian registry had planned their pregnancies according to their DMD stop dates; this is reflected in the significant drop in DMD usage during pregnancy: while 76.7% of the Canadian cohort were on some form of DMD prior to pregnancy only 22.2% were exposed in pregnancy. In contrast 85.3% of the German cohort continued DMDs in pregnancy. Hence the longer interruption of treatment in the CAN group may have been a personal choice rather than an indicator of less severe disease. Also the similarities in disease duration, age at MS diagnosis, and relapse frequency at conception between both groups further confirms that disease severity was likely comparable between the two cohorts.

The shorter follow-up duration postpartum, smaller sample size and the repeated participation of seven of the participants with two different pregnancies in the CAN group may have biased our findings. The follow-up durations were shorter in the CAN than GER, which received follow-up for approximately 3 months longer (Table 2). Postpartum relapses, however, typically occur in the first 3-6 months after delivery and hence our primary outcome, time to first postpartum relapse is unlikely to have been affected. While the German registry enrolled only women who were already pregnant, the Canadian registry had enrolled several women who were in the planning
stages of pregnancy, six of whom did not conceive by the time this study was conducted and hence were censored. An additional eight women were not reached for the final interview and their outcomes could not be determined and they also had to be censored.

Only 68 women (89 pregnancies total) completed all four interviews. We acknowledge that this small number may have rendered the study underpowered to detect a true difference.

While dietary sources may contribute to calcidiol status, this information was not collected from each cohort. Most published studies argue, however, that dietary sources of Vitamin D, even with fortification of dairy products, fail to yield serum calcidiol levels achievable by supplementation. In Germany food fortification is not mandatory and usage of fortified food is not recommended for the general population. In contrast in Canada, many of dairy products are fortified with Vitamin D. Hence it is likely that in addition to the supplemental intake of Vitamin D the dietary intake of Vitamin D in the CAN women may have also been higher, widening the serum calcidiol gap between the two cohorts.

Latitude and climate differences are also important determinants of calcidiol status. Despite the geographical and size difference between the two countries, 68 (83.0%) of the CAN group women resided in Southern Ontario and Quebec; the average latitude: 44°N is comparable to the average Germany’s latitude: 51°N. Both countries have cold winters when UVB is not a significant source of endogenous calcidiol production. However without data on outdoor activities, winter vacationing to sunny
destinations, and tanning bed/sunbathing habits, we cannot rule out
differential subcutaneous calcidiol production between the cohorts.

Known racial differences in Vitamin D metabolism, and genetic
differences in certain alleles, influence serum calcidiol response to
supplementation.\textsuperscript{27} Hence one of the most important differences that could
have influenced our findings is the racial makeup of the CAN cohort.
Despite the ethnic diversity in Canada, 64 (78.0\%) of our CAN group
consisted of European descent women (majority Western European). 10
(12.2\%) were half European, two Indian, one Iranian, one Caribbean, one
far East Asian and two East African and one unclassified. Thus the CAN
group was ethnically much more homogenous than the general Canadian
population and share similarities with the GER group. Furthermore,
restricting the analysis by omitting the non-European descent women in
the CAN group did not change the results (not shown).

7. CONCLUSION

This study compares postpartum disease course in a Vitamin D
supplementing Canadian group and a non-supplementing GER group. We
found no significant difference between groups on disease resurgence
postpartum, in spite of the greater DMD usage in the German cohort. We
observed that significantly more women in the CAN group breastfed and did
so for a longer duration compared to the GER group, most of whom may
have abstained due to concerns about safety of DMDs while breastfeeding.
The prolongation of the gestational remissive phase by a few months
postpartum may not significantly alter long-term disease outcomes, it may however substantially alleviate stress, improve maternal well-being and adjustment to the responsibilities of a newborn. Further studies are required to confirm if Vitamin D can be used as a routine part of MS management in the postpartum period, and to determine the optimal dose that is protective for mom while safe for the breastfeeding infant.
Table 1: Comparison of Maternal Characteristics between Cohorts

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Canadian</th>
<th>German</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy (number)</td>
<td>82</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Height (meters) Mean±SD</td>
<td>1.67 ± 0.06</td>
<td>1.69 ± 0.06</td>
<td>0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-Pregnancy Weight (kg) Mean ± SD</td>
<td>67.5 ± 16.2</td>
<td>68.5 ± 14.4</td>
<td>0.645&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-Pregnancy BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;) Mean±SD</td>
<td>24.3 ± 5.5</td>
<td>23.9 ± 4.6</td>
<td>0.542&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age at conception (years) Mean±SD</td>
<td>32.6 ± 3.9</td>
<td>33.1 ± 4.0</td>
<td>0.309&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Obstetric History Median [IQR:25%-75%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graviа</td>
<td>1 [1-2]</td>
<td>2 [2-2]</td>
<td>&lt;0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parity</td>
<td>1 [1-2]</td>
<td>2 [1-2]</td>
<td>&lt;0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>0 [0-1]</td>
<td>0 [0-1]</td>
<td>0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Therapeutic Abortions</td>
<td>0 [0-0]</td>
<td>0 [0-0]</td>
<td>0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Highest Level of Education</td>
<td>n[%]</td>
<td></td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>High School</td>
<td>6 [7.3]</td>
<td>4 [5.6]</td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>15 [18.3]&lt;sup&gt;*&lt;/sup&gt;</td>
<td>45 [62.5]&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>29 [35.4]</td>
<td>23 [31.9]</td>
<td></td>
</tr>
<tr>
<td>MSc and Higher</td>
<td>32 [39.0]&lt;sup&gt;^&lt;/sup&gt;</td>
<td>0 [0.0]&lt;sup&gt;^&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gestational Age at Recruitment (weeks)</td>
<td>Median [IQR:25%-75%]</td>
<td>12.7 [6.6-26.4]</td>
<td>13.4 [7.1-25.6]</td>
</tr>
<tr>
<td>Postpartum Age at Last Follow-up (months)</td>
<td>Mean ± SD</td>
<td>11.8 ± 6.5</td>
<td>14.6 ± 7.9</td>
</tr>
<tr>
<td>Age at Diagnosis (years) Mean ± SD</td>
<td>26.2 ± 4.8</td>
<td>25.6 ± 5.4</td>
<td>0.420&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Disease duration at conception (years)</td>
<td>Mean ± SD</td>
<td>6.4 ± 4.4</td>
<td>7.6 ± 4.8</td>
</tr>
<tr>
<td>Vitamin D supplement in pregnancy (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>109</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Student’s t-test  
<sup>b</sup> Mann-Whitney U-test;  
<sup>c</sup> Chi-Square Test (* * pairs with the same symbol indicate proportions that differ significantly as per Bonferroni correction)
Table 2a. DMD Exposure Prior to Conception

<table>
<thead>
<tr>
<th>DMD Type</th>
<th>Canadian</th>
<th>German</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NONE</td>
<td>21(23.6%)</td>
<td>3(2.8%)</td>
<td></td>
</tr>
<tr>
<td>2. Glatiramer Acetate</td>
<td>24(27.0%)</td>
<td>22(20.2%)</td>
<td></td>
</tr>
<tr>
<td>3. Interferon Beta-1a</td>
<td>25(28.1%)</td>
<td>49(45.0%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>4. Interferon Beta-1b</td>
<td>6(6.7%)</td>
<td>15(13.8%)</td>
<td></td>
</tr>
<tr>
<td>5. Dimethyl Fumarate</td>
<td>3(3.4%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>6. Fingolimod</td>
<td>6(6.7%)</td>
<td>2(1.8%)</td>
<td></td>
</tr>
<tr>
<td>7. Natalizumab</td>
<td>4(4.5%)</td>
<td>14(12.8%)</td>
<td></td>
</tr>
<tr>
<td>8. IVIG</td>
<td>0(0.0%)</td>
<td>3(2.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2b: DMD Exposure in Pregnancy and Postpartum**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Canadian</th>
<th>German</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD Exposure In pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (22.2%)</td>
<td>93(85.3%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>No</td>
<td>63(77.8%)</td>
<td>16(14.7%)</td>
<td></td>
</tr>
<tr>
<td>Type of DMD Exposures in pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Glatiramer Acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Interferon Beta-1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Interferon Beta-1b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Dimethyl Fumarate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Fingolimod</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Natalizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. IVIG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of DMD exposure in pregnancy (weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25%-75%)</td>
<td>5.0 (3.8-7.1)</td>
<td>4.4(3.6-7.7)</td>
<td>0.774c</td>
</tr>
<tr>
<td>DMD initiated postpartum?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37(50.0%)</td>
<td>103(98.1%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>No</td>
<td>37(50.0%)</td>
<td>2(1.9%)</td>
<td></td>
</tr>
<tr>
<td>Type of DMD initiated postpartum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Glatiramer</td>
<td></td>
<td></td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>2. Interferon Beta-1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Interferon Beta-1b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Dimethyl Fumarate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Fingolimod</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Natalizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Teriflunomide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. IVIG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. New Pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postpartum Age at DMD re-initiation (months) Median (25%-75%)</td>
<td>4.0(1.0-11.2)</td>
<td>2.8(0.6-7.7)</td>
<td>0.132c</td>
</tr>
<tr>
<td>Duration of DMD interruption (months) Median (25%-75%)</td>
<td>25.7(15.6-42.8)</td>
<td>10.6 (8.5-16.3)</td>
<td>&lt;0.001c</td>
</tr>
</tbody>
</table>

*a* Chi-Square Test; 
*b* Fisher’s Exact Test; **Bolded** pairs indicate proportions that differ significantly after Bonferroni correction; 
*c* Mann-Whitney U-test
Table 3. Breastfeeding Behavior

<table>
<thead>
<tr>
<th>Details</th>
<th>Canadian</th>
<th>German</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>n=70</td>
<td>n=102</td>
<td>0.003*</td>
</tr>
<tr>
<td>Yes</td>
<td>62 (88.6%)</td>
<td>70 (68.6%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (11.4%)</td>
<td>32 (31.4%)</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding Duration (months)</td>
<td>n=68</td>
<td>n=95</td>
<td>0.000^</td>
</tr>
<tr>
<td>Median (25%-75%)</td>
<td>6.3(2.8-11.3)</td>
<td>1.8(0-6.9)</td>
<td></td>
</tr>
<tr>
<td>Exclusive Breastfeeding Duration</td>
<td>n=70</td>
<td>n=96</td>
<td>0.006^</td>
</tr>
<tr>
<td>(months)</td>
<td>4.0 (0.5-6.0)</td>
<td>1.6 (0.0-4.8)</td>
<td></td>
</tr>
<tr>
<td>Lactational Amenorrhea</td>
<td>n=55</td>
<td>n=75</td>
<td>0.005^</td>
</tr>
<tr>
<td>Median (25%-75%)</td>
<td>5.0 (3.0-10.4)</td>
<td>3.0 (1.9-6.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi Square Test; ^ Mann Whitney U-test

Table 4. Relapse Details Prior to and During Pregnancy

<table>
<thead>
<tr>
<th>Number of Relapses 18-month pre-pregnancy</th>
<th>Canadian</th>
<th>German</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33 (43.4%)</td>
<td>53 (48.6%)</td>
<td>0.642*</td>
</tr>
<tr>
<td>1</td>
<td>27 (35.5%)</td>
<td>39 (35.8%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12 (15.8%)</td>
<td>14 (12.8%)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>4 (5.3%)</td>
<td>3 (2.7%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Relapses During pregnancy</th>
<th>Canadian</th>
<th>German</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53 (71.6%)</td>
<td>91 (83.5%)</td>
<td>0.019*</td>
</tr>
<tr>
<td>1</td>
<td>17 (23.0%)</td>
<td>18 (16.5%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4 (5.4%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher’s Exact Test; bolded pairs differ significantly
^ Chi Square Test

Table 5. Details of Primary Outcome: Postpartum Relapses

<table>
<thead>
<tr>
<th>Postpartum Relapses</th>
<th>Canadian</th>
<th>German</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of women relapsing</td>
<td>28(41.2%)</td>
<td>63(57.8%)</td>
<td>0.044^</td>
</tr>
<tr>
<td>Relapse Free (N %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 Relapse (N %)</td>
<td>40(58.8%)</td>
<td>46(42.2%)</td>
<td></td>
</tr>
<tr>
<td># of postpartum Relapses</td>
<td>30 (75.0%)</td>
<td>29 (63.0%)</td>
<td>0.639*</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8 (20.0%)</td>
<td>11 (23.9%)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>2 (5.0%)</td>
<td>6 (13.1%)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher’s Exact Test; bolded pairs differ significantly
^ Chi Square Test
Table 6. Adjusted and Unadjusted Hazard Ratio in CAN and GER Cohorts

<table>
<thead>
<tr>
<th>Cohort Category</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>p-value</th>
<th>Nadj</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Included</td>
<td>1.57 (1.03-2.41)</td>
<td>0.036</td>
<td>158</td>
<td>1.11 (0.69-1.78)</td>
<td>0.673</td>
</tr>
<tr>
<td>BMI&lt;25</td>
<td>1.24 (0.74-2.09)</td>
<td>0.420</td>
<td>105</td>
<td>0.83 (0.46-1.49)</td>
<td>0.528</td>
</tr>
<tr>
<td>≥20 weeks of fall/winter pregnancy</td>
<td>2.14 (1.146-4.01)</td>
<td>0.017</td>
<td>71</td>
<td>0.87 (0.37-2.07)</td>
<td>0.754</td>
</tr>
<tr>
<td>BMI &lt;25 &amp; fall/winter duration &lt;19 weeks</td>
<td>0.78 (0.38-1.61)</td>
<td>0.507</td>
<td>57</td>
<td>0.67 (0.32-1.46)</td>
<td>0.328</td>
</tr>
<tr>
<td>Disease Free In Pregnancy</td>
<td>1.73 (1.04-2.88)</td>
<td>0.035</td>
<td>123</td>
<td>1.43 (0.83-2.45)</td>
<td>0.194</td>
</tr>
<tr>
<td>Early DMD Initiation Post-partum (≤2 months)</td>
<td>1.51 (0.59-3.91)</td>
<td>0.393</td>
<td>48</td>
<td>0.67 (0.17-2.66)</td>
<td>0.569</td>
</tr>
<tr>
<td>Late DMD initiation(≥6months)</td>
<td>2.69 (1.34-6.56)</td>
<td>0.007</td>
<td>46</td>
<td>2.15 (0.81-5.70)</td>
<td>0.126</td>
</tr>
<tr>
<td>Exclusive Breastfeeding ≥2months</td>
<td>1.75 (1.00-3.07)</td>
<td>0.049</td>
<td>89</td>
<td>1.38 (0.77-2.49)</td>
<td>0.276</td>
</tr>
</tbody>
</table>

a Adjusted for: age at conception, pregnancy relapses, breastfeeding duration
b Adjusted for: age at conception and breastfeeding duration
c Adjusted for age at conception, pregnancy relapses

Supplementary Table 1: Vitamin D intake in the Canadian cohort (International Units/Day)

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Mean±Sd</th>
<th>Median (25-75%)</th>
<th>Range (Min-Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>first trimester</td>
<td>2686 ± 2020</td>
<td>2375(850-4250)</td>
<td>0-9166</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>2767 ± 2003</td>
<td>2500(1250-4250)</td>
<td>0-9166</td>
</tr>
<tr>
<td>Third trimester</td>
<td>2794 ± 1902</td>
<td>2800(1250-4303)</td>
<td>0-7800</td>
</tr>
<tr>
<td>All trimesters</td>
<td>2749 ±1966</td>
<td>2422(1043-4250)</td>
<td>0-9166</td>
</tr>
</tbody>
</table>

8. Sub Group Analysis

First we restricted the analysis to women with pre-pregnancy BMIs less than 25. The unadjusted risk in Canadian women was 1.24(0.74-2.09) with p=0.420. After adjusting for maternal age, relapses during pregnancy and breastfeeding duration, 105 women in total fit this criteria. 56(44.8%) had a relapse and 49 (39.2%) were censored. Adjusted risk for the CAN group was 0.83(0.46-1.49), a 17% reduction in risk which was not statistically
significant \( p=0.53 \).

Repeating the same analysis for women with BMI \( \geq 25 \) (not shown), demonstrated a near 3 fold increase in risk for CANs in the unadjusted model \( 2.83(1.25-6.42) \ p=0.013 \). After adjusting, 45 women with higher BMIs fit the model (19 CANs and 26 GERs) and the risk while increased in the CANs was no longer statistically significant \( 2.5(0.97-6.51) \ p=0.058 \).

Seasonal fluctuation of serum calcidiol is a well-known phenomenon with lower serum levels during fall and winter months especially in countries at higher latitude. We analyzed risk in women with 20 or more weeks of pregnancy spent during the fall and winter months. The unadjusted risk for relapse was \( 2.14(1.15-4.01) \) in the CAN group and this was significant \( p=0.017 \). After adjusting 71 women fit the criteria (26 CANs and 45 GERs); 38(48.1\%) had a relapse and 33(41.8\%) of women were censored. The risk in CANs fell to \( 0.87(0.37-2.07) \), a 13\% reduction which was not significant \( p=0.75 \).

Repeating the same analysis for women with shorter duration of fall winter pregnancies (<20 weeks) revealed a slight but non-significant risk in the CANs in both the unadjusted and adjusted models [Unadjusted HR: \( 1.16(0.642-2.10) \ p=0.622; \) Adjusted HR: \( 1.01(0.55-1.85) \ p=0.97 \)]. 87 women in total fit the criteria for this analysis (38 CANs and 49 GERs).

Given the apparent role BMI and duration of fall/winter played in the unadjusted models above, we performed a further stratification to women with low BMIs (<25) and shorter winter/fall pregnancies (<20 weeks). The
unadjusted risk in CANs was 0.78(0.38-1.61) p=0.51. After adjusting 57 women in total fit this criteria (27 CANs and 30 GERs). The adjusted risk was 0.67(0.32-1.46) in the CANS, a 33% reduction in risk which was not significant p=0.33.

We then stratified women according to relapse during pregnancy. Since frequency of relapses during pregnancy was a predictor for postpartum risk, we isolated the analysis to women who were relapse free during pregnancy. The unadjusted risk of 1.73(1.04-2.88) with p=0.035 was observed for the CAN cohort. After adjusting for maternal age at conception and duration of breastfeeding, a total of 123 women fulfilled this criteria (45 CANs and 78 GERs); 60 (41.7%) had postpartum relapse and 63(43.8%) were censored. The adjusted hazard ratio in the CAN group was 1.43(0.83-2.45) with p=0.194.

We then stratified women according to their DMD usage postpartum. First we restricted the analysis to women with early DMD initiation, within two months of delivery. The unadjusted risk for CANs was 1.51(0.59-3.91) p=0.39. After adjusting for all three covariates, only 10 CANs and 38 GERs fit this model. The adjusted risk was 0.67(0.17-2.66) p=0.57.

Repeating the analysis for women with delayed DMD therapy (6 months or later after delivery), revealed a 2.96(1.34-6.56) risk in Canadians which was significant p=0.007. After adjusting, a total of 46 women fit the model (14 CANs and 32 GERs) 25 (47.2%) had a relapse and 21(39.6%) were censored. The adjusted HR was 2.15(0.81-5.70) in CANs and no longer
significant (p=0.126).

To investigate the impact of exclusive breastfeeding (i.e. no supplemental feeding), as it was reported to be protective in a recent study, we stratified women according to exclusive breastfeeding duration. We first restricted the analysis to women who exclusively breastfed for at least two months. The unadjusted hazard ratio in CANs was 1.75(1.003-3.07) p=0.049. We adjusted the risk in two ways, first by including breastfeeding duration in the model and second by omitting it. The adjusted hazard ratio when only maternal age and pregnancy relapses were included was 1.58(0.89-2.80) p=0.12. The adjusted hazard ratio when all three covariates were included was 1.38(0.77-2.49) P= 0.28 in CANs. 89 women in total fit this criteria (45 CANs and 44 GERs); 51(54.3%) had a relapse and 38(40.4%) were censored.

Repeating the analysis for women who exclusively breastfed for less than two months demonstrated an unadjusted risk of 0.95(0.44-2.06) p=0.89.

After adjusting, 69 women (19 CANs and 50 GERs) fit the model 31(43.1%) had a relapse and 38(52.8%) were censored. The adjusted hazard ratio in CANs was 0.94(0.38-2.33) and was not significant p=0.90.

When adjusting for just maternal age and relapses in pregnancy the hazard ratio in CANs was 0.63(0.17-2.27) and still not significant p=0.47.

Finally a subgroup of women breastfed while on a DMD. The unadjusted risk in CAN was: 1.13(0.39-3.25) p=0.827. After adjusting 23 women in total fulfilled the criteria (12 CANs and 11 GERs); 14(60.9%) had a relapse and 9(39.1%) were censored. The adjusted risk was 0.61(0.17-2.23) p=0.46.
in the CAN group. 10/12 CANs and 10/11 of GERs breastfed while on a first-line DMD. There was one fingolimod and one tysabri and breastfeeding exposure in the CAN group and one IVIG and breastfeeding exposure in the GER Group. No significant differences were detected between groups on type of DMD usage while breastfeeding. Fisher Exact p=1.000.

9. References


9. Soilu-Hanninen M, Aivo J, Lindstrom BM, et al. A randomised, double blind, placebo controlled trial with Vitamin D3 as an add on treatment to interferon beta-


CHAPTER 4
MANUSCRIPT
THREE

High Dose Gestational Vitamin D Supplementation and Neonatal Outcomes in Women with Multiple Sclerosis

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Funding

The Motherisk MS Pregnancy Registry was funded by Novartis

Authors Contribution

Neda Ebrahimi, M.Sc.: Study concept and design, data acquisition, analysis, interpretation, drafting and revision of manuscript.

Ann Yeh, MD: Study concept and design, critical revision of manuscript for intellectual content, analysis and interpretation
I. ABSTRACT

BACKGROUND

Vitamin D is important for fetal bone development, and its gestational deficiency has been linked to adverse maternal and fetal outcomes. Due to the association between low Vitamin D status and enhanced disease progression of multiple sclerosis (MS), some women with MS take high dose Vitamin D even during pregnancy. However, fetal safety of high dose Vitamin D remains to be shown. Our objective was to compare the outcomes of neonates born to women with relapsing remitting MS, who took ≥4000IU/day of Vitamin D, to a disease matched group with lower doses.

METHODS

Data from women enrolled in a MS pregnancy registry was utilized. The women were categorized into two groups: High Dose (HD) (≥4000 IU/day) and Low Dose (LD) (<4000 IU/day) groups. The two groups were compared
on the following outcomes: birth weight and birthweight percentiles (BW%) adjusted for infant’s sex, gestational age at birth, and maternal race (primary outcomes). Secondary outcomes were rates of miscarriage, delivery mode, gestational age at birth, major birth defect rates, preterm birth rates (<37 weeks), low birth weight rates (<2500 grams), head circumference and length at birth.

RESULTS

80 pregnancies (51 in LD and 29 in HD group) were analyzed. The median Vitamin D dosage throughout pregnancy was 1400 (IQR: 400-2294) IU/day and 4400 (IQR: 4250-5417) IU/day in the LD and HD groups respectively.

Head circumference was significantly higher in the HD group (35.0 ± 0.9 cm vs. 33.8 ± 1.7 cm: p=0.012).

There were no significant differences between HD and LD on gestational age at birth, birth weight and length, and BW% adjusted for maternal BMI, average Vitamin D dose and duration of fall winter pregnancy.

There was a significant inverse relationships between third trimester Vitamin D dose and BW% (Pearson’s r=-0.245, p=0.048).

CONCLUSION

Exceeding the current Health Canada recommendations of 4000 IU/day of Vitamin D in an MS cohort was not associated with any increased risk for adverse outcomes in neonates. Future studies with larger cohorts and serum measurements are required to determine the precise effect of such doses on neonatal outcomes.
2. **BACKGROUND**

As a multipotent steroid, Vitamin D is involved in not only calcium and phosphate homeostasis, but also multiple cellular systems and signaling pathways, with nearly 10% of the human genome under its influence.\(^1\) The link between Vitamin D status and the immune system is of particular interest when considering the role of Vitamin D in disease manifestations and course in multiple sclerosis (MS). Many immune cells contain receptors for an active form of Vitamin D metabolite (1,25(OH)\(_2\)D), which is known to exert anti-proliferative and anti-inflammatory effects.\(^2\) Lower serum Vitamin D levels have been observed in patients with Relapsing Remitting MS (RRMS) especially those with more progressive disease, and those with Secondary Progressive MS (SPMS).\(^3\) MS patients with lower levels of serum calcidiol, a precursor of the active form, are reported to experience a higher relapse rate; an inverse relationship exists between serum calcidiol and MS relapse rates.\(^4,5\) Furthermore, serum calcidiol levels decline during a relapse and tend to be lower compared to between-relapse states.\(^6\)

Anecdotal reports regarding the benefits of Vitamin D as an immunomodulator has led to different practices in Vitamin D usage around the world.\(^7,8\) Currently there is no consensus on optimal serum levels of Vitamin D, but most experts agree that levels below 50 nmol/l should be corrected.\(^9\) In human pregnancies, Vitamin D is vital in all stages of gestation.\(^10,11\) Vitamin D deficiency has been associated with a number of neonatal complications including growth retardation (small for gestational age.
or low birth weight), respiratory illnesses, eczema and food allergies.\textsuperscript{9,12,13} Despite pregnancy being a risk factor for hypovitaminosis D, no universal guidelines exist for optimal dosage and serum levels, and recommendations range from 400 IU/day to a maximum 4000 IU/day in different parts of the world.\textsuperscript{7} Studies on cardiovascular disease and cancer suggest serum levels above 75-100 nmol/L to be optimal.\textsuperscript{4} A dose of 4000 IU/day for several weeks followed by a 2000 IU/day maintenance dose thereafter may achieve these levels.\textsuperscript{4} The current Health Canada recommendation for pregnant and/or breastfeeding women is a maximum of 4000 IU/day especially during the fall and winter months when serum calcidiol levels tend to decline.\textsuperscript{14}

Studies on Vitamin D supplementation in pregnant women with MS are lacking, and given a trend toward lower birth weight in babies born to women with MS,\textsuperscript{15} and the immunomodulatory role of Vitamin D, the impact of supplementation in pregnant women with MS is of interest. Through our teratology information service in Toronto, Canada, we encountered pregnant women with RRMS, who have been supplementing regularly with Vitamin D since their MS diagnosis. At the time of pregnancy many of these women had already been supplementing rigorously for several years, and continued to do so during pregnancy, with some women continuing to exceed the recommended 4000 IU/day. Most of these women resumed the same or higher doses during the postpartum period out of fear of experiencing a relapse. In this study, we assessed pregnancy and neonatal outcomes of this unique cohort of women with relatively high doses of Vitamin D supplementation during pregnancy.
3. METHODS

MS pregnancy Registry

We analyzed prospectively collected data in a Canadian pregnancy and MS registry. This registry was constructed in 2011 as part of a multinational study by Novartis to assess pregnancy and neonatal outcomes in women with MS exposed to fingolimod (Gilenya®). The purpose of the Canadian registry was to contribute a disease matched comparison group of pregnant women not exposed to fingolimod in order to compare pregnancy and postpartum disease outcomes.

Women who were already pregnant and who were planning pregnancies were initially either self-referred or referred by a healthcare provider to the teratology information service (Motherisk) at the Hospital for Sick Children in Toronto. Patients with MS calling the service for drug safety information were informed of the MS registry, and those who provided consent were enrolled in the registry. Upon enrollment, patients completed four phone interviews: 1) baseline interview at enrollment; 2) during pregnancy; 3) after delivery; and 4) twelve months after delivery. Detailed data was collected at each interview, which included information on maternal demographics, obstetrics and disease history, exposures during pregnancy including dosage of Vitamin D used, pregnancy, delivery and neonatal outcomes, and health details of neonates at birth. The database consisted of de-identified information, and usage of the data for this study was approved by the
Pregnancies were categorized into High Dose (HD) and Low dose (LD) groups according to the average daily Vitamin D intake in each trimester. For each pregnancy the total daily dose of Vitamin D intake was calculated by adding the Vitamin D in the prenatal vitamin formulations to the additional supplemental dose. The average dose per trimester was calculated by taking the average daily dose (e.g., if in the first trimester a women took 1000 IU/day for 50 days and 1300 IU/day for 40 days her average daily dose in the first trimester would be: (1000 x 50 + 1300 x 40)/90 = 1133 IU/day.) The average dose throughout pregnancy was calculated by taking the means of average daily doses across the three trimesters. Pregnancies, in which an average dose never exceeded 4000 IU/day in any trimester, were categorized into the LD group. If average Vitamin D intake per day was 4000 IU or more in at least one of the trimesters, that pregnancy was categorized into the high-dose HD group.

**Statistical Analysis**

Continuous parametric data was compared between HD and LD groups using Independent Student’s t-test; and Mann Whitney U-test for non-parametric data. Categorical data between groups were compared using Chi Square or Fisher Exact according to data characteristics. To determine the impact of confounders on the primary outcome a multiple regression analysis was performed. Variables that are known to impact serum calcidiol levels and (i.e., maternal BMI, age, duration of fall-winter
pregnancy) were incorporated into the model. Variables that demonstrated a significant relationship to the outcome were adjusted for in an ANCOVA analysis when comparing the two groups. Pearson’s correlation test was conducted to determine if any association between average daily dose per trimester of pregnancy and each of the primary outcomes exists. All analyses were completed using SPSS software version 24.

4. RESULTS

A total of 82 planning or pregnant women were enrolled in the registry. Six women never conceived during the study. 83 pregnancies occurred in the remaining 76 women (7 women became pregnant twice during the study). The exact Vitamin D intake dosage was known in 80 of these 83 pregnancies. The average dose throughout pregnancy ranged from 0 to 9166 IU/day with a median of 2422 IU/day for the entire cohort. The three pregnancies with unknown Vitamin D were omitted from the primary outcome analysis.

Vitamin D Dose

51 pregnancies were categorized into the LD group, and 29 were in to the HD group. The median dose throughout pregnancy was 1400 IU/day (range: 0 – 3800 IU/day) in the LD and 4400 IU/day (range 2624-9166 IU/day) in HD groups. The HD consumed nearly 3000 IU/day more than the LD group at any point in pregnancy. There was a strong correlation between using high doses (≥4000IU/day) in the first trimester and high dose in the
subsequent trimesters of pregnancies (Pearson’s Correlation $r=0.94$ $P<0.001$ for second trimester; $r=0.84$ $p<0.001$ in third trimester). 19/29 (75%) of HD pregnancies were exposed to greater than 4000 IU/day.

**Maternal Characteristics**

No significant differences in height, pre-pregnancy weight, BMI, age at conception, gestational age at recruitment and postpartum follow-up were seen between the groups (Table 1). Similarly, no significant difference was observed in the median number of gravida, parity and spontaneous abortions. There were also no significant differences between the groups in the age at MS diagnosis, number of pre-pregnancy relapses, duration of therapy interruption, nor rates of exposures to disease modifying drugs (DMD) in pregnancy.

**Pregnancy and Neonatal Outcomes**

Of the 80 pregnancies with known Vitamin D doses, outcome data were available from 72 pregnancies. There were 68 live births and 5 miscarriages (Table 2). No stillbirths, terminations, ectopic or molar pregnancies were reported. 45 pregnancies in the LD group lead to 46(92.0%) live births (one set of twins) and 22 of the HD pregnancies lead to 22(95.7%) live singleton births. The rates of live birth and miscarriage were not significantly different. There were no differences in the rates of birth defects, gender, neonatal health complications, preterm births, and low birth weight (<2500g) between groups. While gestational age at birth, birth weight and body length were not significantly different, head circumference was
significantly higher in the HD group (35.0 cm vs. 33.8 cm p=0.012).

**Predictors of Birth Weight**

Multiple regression analysis was run to predict birth weight and birth weight percentiles (BW%) based on maternal BMI, parity, maternal age at conception, duration of winter/fall pregnancy and average dose of Vitamin D in pregnancy. These predictors have been shown in past studies to be associated with birth weight.\(^\text{16-20}\) In our study, none of the variables were significant predictors of birth weight (Table 3). As shown in Table 4 however, average dose of Vitamin D in pregnancy, pre-pregnancy BMI and winter/fall duration of pregnancy significantly predicted BW%: \(F(5.59)=6.482 \ R^2=0.355 \ p<0.001\). All three predictors bear an inverse relationship with BW%, such that higher Vitamin D dose, maternal BMI, and duration of fall/winter pregnancy were associated with lower BW%. A follow-up ANCOVA analysis to compare BW% between the HD and LD groups while adjusting for pre-pregnancy BMI, and winter duration of pregnancy was conducted. No significant difference in BW% between the two groups after adjusting for these covariates was found \(F(3,1)=1.555, \ p=0.217\).

**Vitamin D Dose per Trimester and BW%**

Since average Vitamin D dose significantly predicted BW% in the multiple regression model a follow-up Pearson’s correlation analysis was conducted to determine the association of average dose per trimester of
pregnancy and BW\%s. A weak inverse correlation was found between dosage in the third trimester of pregnancy and BW\%s $r=-0.245$, $p=0.048$.

5. **DISCUSSION**

In this study we report pregnancy and neonatal outcomes in a group of Canadian women with RRMS supplementing with Vitamin D. We compared outcomes between high dose (≥ 4000 IU/day in one or more trimesters) and low dose (<4000IU/day) users. We observed similar pregnancy and neonatal outcomes between the HD and LD groups, except for head circumference, which was significantly larger in the HD group. However head circumference data was available only in a small number of infants, cautioning against over-interpretation of this finding.

Furthermore, additional analysis excluding premature infants, and using head circumference percentiles did not yield statistically significant differences between the groups (supplementary section).

Many maternal and environmental factors may be at play in driving serum Vitamin D levels. In our multiple regression analyses we observed an inverse relationship between pre-pregnancy BMI, Vitamin D dose and winter duration of pregnancy and BW\%s. Seasonal variations of serum calcidiol are known to occur, with a significant decline during the fall and winter months.\textsuperscript{4} Hence women that get pregnant in early fall are typically at a higher risk for Vitamin D deficiency.\textsuperscript{21} Consistent with this we observed that the more weeks of pregnancy spent in winter/fall months, the lower the birth weight and BW\%s. Maternal BMI was positively associated with birth weight in our study and while not statistically significant, an association of
high BMI with higher birth weight is consistent with previously reported data.\textsuperscript{22} The inverse relationship between BMI and average dose of D with BW\% is surprising. Restricting the analysis to women with BMI<25 removed the significance (not shown). It may be that comorbidities as well as lower serum calcidiol levels in some of the overweight women impacted these findings.

There are several strengths and weaknesses which should be addressed. One strength of our cohort was its relative homogeneity. The majority of participants were of European descent; hence the influence of race, ethnicity and skin pigmentation that are known to impact calcidiol levels were not a major source of confounding. The study cohort is quite unique in that many participants supplemented with Vitamin D from the time they were diagnosed with MS as per recommendations of their neurologist. By the time of conception they had already been supplementing daily for several years. Furthermore, 70\% of these women had at least a bachelor degree (38\% had an MSc or higher) and most were very proactive about pursuing healthy lifestyles and conducting their own research on MS. Most of these women were very motivated to take Vitamin D and did so diligently, hence both compliance and accuracy of self-reported data is likely better than the norm.

There is significant variability between individuals’ serum calcidiol response to supplementation; many factors including dosage, duration of treatment, BMI, baseline serum calcidiol, age and season are thought to influence response.\textsuperscript{23} A 2014 study conducted meta-regression on 33 RCTs,
analyzed patient and supplementation factors that impacted serum response to supplementation. Only trials consisting of healthy non-pregnant subjects, supplementing orally with D₃ with a control/placebo group were included. A total of 3659 treated subjects were analyzed; daily dosage ranged from 200-4000IU/day and duration of treatment 1 to 36 months. Higher doses (≥800 IU/day) and longer duration of supplementation (>6 months) were associated with greater pooled mean differences in serum calcidiol levels between treated and controls (~35 and 40nmol/L for dose and duration respectively). However the dose response to Vitamin D appears to follow a curvilinear pattern with a flattening effect at doses above 800IU/day. Individuals with baseline levels below 50nmol/L display a more pronounced response to supplementation. This curvilinear response may vary significantly between individuals and hence a fixed dose may not achieve the desired level for everyone. Hence while serum levels were not monitored in our study, it is probable that given the long term commitment to supplementation, compared to the non-supplementing healthy general population and in particular to other women with MS, most of our women have higher serum calcidiol levels. Data from the 2007–2009 Canadian Health Measures Survey reported mean serum calcidiol levels in non-supplementing Canadians, between ages 20-39 to be 55.7nmol/L in winter (N=336) and 65.9nmol/ in the summer (N=552) months. If we adopt the 35nmol/L change in serum levels reported from Shab Bidar et al, it is possible that many women in our cohort have serum levels 85nmol/l or more. This brazen assumption would have to be confirmed in future studies.
with measured levels.

As we did not study individuals from groups other than Caucasian women, these results may not hold for groups with other racial/ethnic backgrounds. The major limitations of this study however, are the lack of serum monitoring, small sample size, and limited clinical information related to diet. As the original purpose for the registry was not related to Vitamin D supplementation, serum samples were never collected. We did not collect information on dietary intake of Vitamin D particularly oily fish, outdoor activities during summer/spring months, frequency and duration of sunny destination vacations in the fall/winter months and paternal race of the babies.

The size of the cohort, and in particular the small number in the HD group may have led to a type II error. While 83 pregnancies were expected to yield 80% power for this study, given the small difference in the observed birth weights between high and low dose groups and the omission of women lost to follow-up we may need a significantly larger cohort to detect a true difference.

Alternatively, it may be that supplementation with Vitamin D in women with sufficient baseline levels does not clinically impact birth weight, and that the effect on birth weight is significant only in deficient women.

While we acknowledge that these weaknesses limit the applicability and interpretation of our findings, we feel that this study provides a platform for future studies investigating optimal dose of Vitamin D supplementation in
MS pregnancies and provides reassurance to patients who have inadvertently exceeded the maximum recommended dose in pregnancy. While we did see a non-significant prolongation in disease free period postpartum in the HD groups (supplementary), it would be of particular interest to investigate the impact of Vitamin D supplementation on postpartum disease activity and resurgence particularly in women with insufficient serum levels in future studies.

Most Vitamin D studies in pregnancy have focused on correcting deficiency in pregnant mothers (without MS) and subsequent fetal outcomes. Only one small Iranian study with 15 RRMS women supplementing for a few weeks in pregnancy was identified, but the primary outcome of interest was disease course. Our study is unique in its evaluation of high Vitamin D dosage not only in one trimester but throughout pregnancy in a group of women who may have already had sufficient serum calcidiol levels at the start of pregnancy. Through this study we can neither justify nor reject regular high dose supplementation in women with RRMS. Further studies with larger sample sizes and serum level measurement are required to ascertain safety of higher doses. Until such studies are conducted, planning and pregnant women who wish to exceed the recommendations are strongly encouraged to monitor serum levels regularly and to adjust dose accordingly.
6. Supplementary Analysis

Head Circumference

Head circumference data was available in 36 infants (12 in HD and 24 in LD groups). Three infants in the LD group were premature. We repeated the analysis, comparing head circumference between HD and LD by restricting data to infants born after 37 weeks. The mean head circumference was 34.2 ±1.3 cm 35.0 ± 0.9 cm in the LD and HD group respectively; the two tailed t-test was not significant p=0.050. In further exploratory analysis we calculated head circumference percentiles according to infant gender and gestational age at birth using PediTool Fenton 2013 growth calculator. The median head circumference percentiles were 46.0%(IQR 25.8%-60.5%) and 56.5%(IQR: 42.5%-66.3%) in the LD and HD groups respectively; Mann Whitney U-test did not detect a significant difference p=0.188.

Postpartum Relapses

Our sample size and duration of follow-up preclude us from analyzing postpartum disease activity. We briefly describe here our observation of postpartum relapses in these groups.

40 women experienced relapses in the postpartum period. 29/44 (65.9%) in the LD group and 11/22 (50%) in the HD group. The first postpartum relapse occurred at 12.2±10.1 months in the LD group and at 14.4±11.1 months in the HD group. Kaplan Meier analysis found a higher risk in the LD group on
postpartum disease resurgence; unadjusted hazard ratio= 1.40 (0.70-2.81) p=0.342 (not shown). A multiple regression analysis was conducted to identify confounders for postpartum relapse, only maternal age at conception, breastfeeding duration, and pre-pregnancy relapses appeared to significantly predict time to first postpartum relapse F(3,58)=6.67 P=0.001(not shown) . Average dose of Vitamin D, maternal BMI, age at MS diagnosis, disease duration at conception, duration of DMD interruption, duration of winter/fall in pregnancy, and initiation of DMDs in the postpartum period did not predict postpartum relapses. A cox proportional hazard was utilized to adjust for the three predictors. After adjusting for maternal age at conception, breastfeeding duration and pre-pregnancy relapses, an increase in relapse risk was observed in the LD group but this was still not statistically significant: 1.72(0.824-3.569) p=0.149.
Table 1. Comparison of maternal characteristics in the LD and HD groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low Dose Group (N=51)</th>
<th>High Dose Group (N=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Trimester Dose (IU/day)</td>
<td>1250 (400-2250)</td>
<td>4400 (4175-5400)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Second Trimester Dose</td>
<td>1400 (400-2450)</td>
<td>4441 (4250-5400)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Third Trimester Dose</td>
<td>1400 (400-3250)</td>
<td>4750 (4263-5400)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Throughout All Trimesters Dose</td>
<td>1400 (400-2294)</td>
<td>4400 (4250-5417)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Gestational Age at recruitment (weeks)</td>
<td>13.1 (7.7-27.9)</td>
<td>15.1 (8.0-26.9)</td>
<td>0.952*</td>
</tr>
<tr>
<td>Age at last Follow-up (mo)</td>
<td>12.7 ± 6.2</td>
<td>9.9 ± 7.1</td>
<td>0.124^</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 ± 0.07</td>
<td>1.68 ± 0.05</td>
<td>0.138^</td>
</tr>
<tr>
<td>Pre-Preg Weight (kg)</td>
<td>66.6 ± 15.7</td>
<td>67.0 ± 13.7</td>
<td>0.925^</td>
</tr>
<tr>
<td>Pre-Preg BMI</td>
<td>24.2 ± 5.2</td>
<td>23.7 ± 4.7</td>
<td>0.687^</td>
</tr>
<tr>
<td>Age at conception (yr)</td>
<td>32.5 ± 3.8</td>
<td>32.9 ± 4.1</td>
<td>0.685^</td>
</tr>
<tr>
<td>Gravida</td>
<td>0.0 (0.0-2.0)</td>
<td>1.0 (0.0-1.0)</td>
<td>0.811*</td>
</tr>
<tr>
<td>Parity</td>
<td>0.0 (0.0-1.0)</td>
<td>0.0 (0.0-1.0)</td>
<td>0.632*</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-1.0)</td>
<td>0.362*</td>
</tr>
<tr>
<td>Age at MS Diagnosis (yrs)</td>
<td>25.8 ± 4.5</td>
<td>27.1 ± 5.7</td>
<td>0.334^</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>6.4 (3.3-8.3)</td>
<td>3.6 (2.4-7.8)</td>
<td>0.247*</td>
</tr>
<tr>
<td>Pre-pregnancy relapse</td>
<td>1.0 (0.0-1.0)</td>
<td>1.0 (1.0-2.0)</td>
<td>0.745*</td>
</tr>
<tr>
<td>DMD interruption (mo.)</td>
<td>24.9 (16.2-47.5)</td>
<td>32.5 (15.6-37.0)</td>
<td>0.945*</td>
</tr>
<tr>
<td>DMD Exposures in preg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (19.6%)</td>
<td>8 (27.6%)</td>
<td>0.578~</td>
</tr>
<tr>
<td>No</td>
<td>41 (80.4%)</td>
<td>21 (72.4%)</td>
<td></td>
</tr>
</tbody>
</table>

*Mann Whitney U test;  
^Student’s independent t-test;  
~ X² test
Table 2: Pregnancy, Delivery and Neonatal Outcomes in the HD and LD Groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>LD (&lt;4000 IU/day) N=49</th>
<th>HD (≥4000 IU/day) N=23</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall/Winter Duration (weeks)</td>
<td>20.0±5.4</td>
<td>19.2±5.4</td>
<td>0.570^</td>
</tr>
<tr>
<td>Birth Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live Birth</td>
<td>46(92.0%)</td>
<td>22(95.7%)</td>
<td></td>
</tr>
<tr>
<td>Miscarriage</td>
<td>4(8.0%)</td>
<td>1(4.3%)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>42(91.3%)</td>
<td>21(95.5%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4(8.7%)</td>
<td>1(4.5%)</td>
<td></td>
</tr>
<tr>
<td>Neonatal Health Issues</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35(76.1%)</td>
<td>18(81.8%)</td>
<td>0.758~</td>
</tr>
<tr>
<td>Yes</td>
<td>11(23.9%)</td>
<td>4(18.2%)</td>
<td></td>
</tr>
<tr>
<td>Delivery Mode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>25(55.6%)</td>
<td>17(77.3%)</td>
<td>0.234~</td>
</tr>
<tr>
<td>C/Section Planned</td>
<td>7(15.6%)</td>
<td>1(4.5%)</td>
<td></td>
</tr>
<tr>
<td>C/Section Emergency</td>
<td>13(28.9%)</td>
<td>4(18.2%)</td>
<td></td>
</tr>
<tr>
<td>Gestational Age at Birth</td>
<td>38.8±1.6</td>
<td>39.3±1.9</td>
<td>0.223^</td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>40(87.0%)</td>
<td>21(95.5%)</td>
<td>0.414~</td>
</tr>
<tr>
<td>Yes</td>
<td>6(13.0%)</td>
<td>1(4.5%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28(60.9%)</td>
<td>15(68.2%)</td>
<td>0.602^</td>
</tr>
<tr>
<td>Male</td>
<td>18(39.1%)</td>
<td>7(31.8%)</td>
<td></td>
</tr>
<tr>
<td>Birth Weight (grams)</td>
<td>3256.1 ± 463.9</td>
<td>3275.8 ± 434.0</td>
<td>0.868^</td>
</tr>
<tr>
<td>BW%</td>
<td>46.3(32.8-72.1)</td>
<td>43.7(23.3-69.7)</td>
<td>0.521*</td>
</tr>
<tr>
<td>Low (&lt;2500g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>44(95.7%)</td>
<td>21(95.5%)</td>
<td>1.000~</td>
</tr>
<tr>
<td>Yes</td>
<td>2(4.3%)</td>
<td>1(4.5%)</td>
<td></td>
</tr>
<tr>
<td>Length at Birth (cm)</td>
<td>50.7 ± 2.7</td>
<td>51.2±2.7</td>
<td>0.505^</td>
</tr>
<tr>
<td>Head Circumference (cm)</td>
<td>33.8 ± 1.7</td>
<td>35.0 ± 0.9</td>
<td>0.012^</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38(84.4%)</td>
<td>22(100%)</td>
<td>0.086~</td>
</tr>
<tr>
<td>No</td>
<td>7(15.6%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding Duration</td>
<td>6.0(2.7-10.9)</td>
<td>7.1(3.1-12.6)</td>
<td>0.374*</td>
</tr>
<tr>
<td>Exclusive Breastfeeding Duration</td>
<td>4.4(0.8-6.0)</td>
<td>3.2(1.2-6.0)</td>
<td>0.497*</td>
</tr>
</tbody>
</table>

*Mann Whitney U test; ^Student’s independent t-test; ~ Fisher Exact test; X Pearson’s chi-Square
### Table 3. Multiple Regression Analysis for Predictors of Birth Weight

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta</th>
<th>Standard Error B</th>
<th>Beta (standardized)</th>
<th>p-value</th>
<th>95% CI (lower bound, Upper Bound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>3860.86</td>
<td>535.39</td>
<td></td>
<td>0.000</td>
<td>2789.56, 4932.17</td>
</tr>
<tr>
<td>Average Dose</td>
<td>-0.01</td>
<td>.03</td>
<td>-0.04</td>
<td>0.77</td>
<td>-0.07, 0.05</td>
</tr>
<tr>
<td>Age at Conception</td>
<td>-14.73</td>
<td>14.97</td>
<td>-0.13</td>
<td>0.33</td>
<td>-44.67, 15.22</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>3.94</td>
<td>11.21</td>
<td>+0.05</td>
<td>0.73</td>
<td>-18.49, 26.36</td>
</tr>
<tr>
<td>Parity</td>
<td>102.73</td>
<td>88.68</td>
<td>+0.15</td>
<td>0.25</td>
<td>-74.73, 280.18</td>
</tr>
<tr>
<td>Winter/Fall Duration</td>
<td>-11.09</td>
<td>11.00</td>
<td>-0.13</td>
<td>0.32</td>
<td>-33.09, 10.92</td>
</tr>
</tbody>
</table>

\[ F(5, 59) = 0.728 \ P = 0.605 \ R^2 = 0.058 \]

### Table 4. Multiple Regression Table for predictors of BW\% (adjusted for GA at birth, maternal race and gender)

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta</th>
<th>Standard Error B</th>
<th>Beta (standardized)</th>
<th>p-value</th>
<th>95% CI (lower bound, Upper Bound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>149.59</td>
<td>25.07</td>
<td></td>
<td>0.000</td>
<td>99.42, 199.42</td>
</tr>
<tr>
<td>Average Dose</td>
<td>.00</td>
<td>.00</td>
<td>-0.29</td>
<td>0.009</td>
<td>-0.01, 0.00</td>
</tr>
<tr>
<td>Age at Conception</td>
<td>-.74</td>
<td>.70</td>
<td>-0.12</td>
<td>0.293</td>
<td>-2.15, 0.66</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>-1.48</td>
<td>.52</td>
<td>-0.31</td>
<td>0.007</td>
<td>-2.53, -0.43</td>
</tr>
<tr>
<td>Parity</td>
<td>5.94</td>
<td>4.15</td>
<td>+0.15</td>
<td>0.158</td>
<td>-2.37, 14.25</td>
</tr>
<tr>
<td>Winter/Fall Duration</td>
<td>-1.57</td>
<td>.52</td>
<td>-0.33</td>
<td>0.003</td>
<td>-2.60, -0.54</td>
</tr>
</tbody>
</table>

Dependent Variable: % \[ F(5, 59) = 6.482 \ R^2 = 0.355 \ P < 0.001 \]

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Table 5. Association between average dose per trimester and BW%

<table>
<thead>
<tr>
<th>Model</th>
<th>First trimester</th>
<th>Second Trimester</th>
<th>Third trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation R</td>
<td>-0.186</td>
<td>-0.216</td>
<td>-0.245</td>
</tr>
<tr>
<td>p-value</td>
<td>0.131</td>
<td>0.080</td>
<td>0.048</td>
</tr>
<tr>
<td>N</td>
<td>67</td>
<td>67</td>
<td>66</td>
</tr>
</tbody>
</table>

7. References


23. Shab-Bidar S, Bours S, Geusens PP, Kessels AG, van den Bergh JP.


CHAPTER 5
DISCUSSION AND CONCLUSION

Summary of Finding

We assessed pregnancy, neonatal and disease outcomes for two commonly used therapeutic agents in MS, natalizumab and Vitamin D, and found no significant increase in adverse outcomes with either product. The three manuscripts in this thesis provide important information on common exposures in a marginalized group of MS patients—pregnant women—selectively excluded from most studies, due to ethical implications regarding the developing fetus. This sidelining has led to significant deficit in managing RRMS, known to undergo dynamic shifts from the pre-conception period up to one year after delivery. Hence many treatments proven to be efficacious in non-pregnant cohorts, may not be viable options in planning and breastfeeding women. Here we concentrate on two such treatments, neither of which have been adequately studied, and contribute preliminary findings on their safety and/or effectiveness in this particular population. This thesis assesses gestational exposure to two commonly used MS therapies—one, a second line disease modifying drug and the other, a vitamin supplement, with a focus on pregnancy, neonatal and disease outcomes.
Discussion

In the first manuscript we compared 102 pregnancy outcomes in a cohort of German women with exposure to natalizumab in the first trimester of pregnancy to a disease matched group with 95 pregnancies, 25 of which were exposed to first line DMDs. The outcomes of both MS cohorts were also compared to 97 healthy women matched to the exposed group on BMI, age at conception and gestational age at recruitment and with no exposure to any teratogens in pregnancy.

Both MS cohorts had a significantly higher rate of miscarriage and lower mean birth weight and birth length compared to healthy controls, but they did not differ significantly from each other. The rates of birth defects and other adverse neonatal outcomes such as low birth weight (<2500 grams), premature birth (<37 weeks) and reduced head circumference, were similar between the three groups, suggesting that early exposure to natalizumab is not expected to increase the risk for adverse outcomes. The presence of a disease matched group is significant as it allowed us to separate disease impact from exposure effect on outcomes. Without it, the higher rate of miscarriage, lower birth weight and birth length may have been ascribed to natalizumab exposure alone.

Previously published studies on gestational exposure to natalizumab, which were briefed in the introduction of this thesis, are limited to manufacturer’s pregnancy registry\textsuperscript{70} and accidental exposures and cases.\textsuperscript{92} None included a disease matched comparison group.
While the majority of published reports on neonatal outcomes in women with MS are reassuring, some reports have described an association between MS itself and negative outcomes such as lower birth weight, higher miscarriages, cesarean sections and assisted vaginal deliveries.\textsuperscript{39,48} Some of the adverse outcomes appear to be more frequent in DMD treated versus DMD naïve women and those with more established disease.\textsuperscript{48,93} As natalizumab is a second line treatment for patients with active disease, some of the adverse outcomes observed in natalizumab exposed pregnancies may be attributed to disease severity. This necessitates the presence of a disease matched comparison group to account for the role of disease itself. In our study, which is the first and largest controlled study on natalizumab pregnancy exposures, we addressed this need using a disease matched group. However in-spite of similarities between groups, as EDSS scores and other objective clinical measures were not available, we were not able to match for disease severity, a major limitations of our study. Also the healthy control group used in our study consisted of women voluntarily calling the Motherisk service line for treatment and management of moderate to severe nausea and vomiting of pregnancy, a selection bias that may have driven our observation of higher miscarriages and lower birth weights in both MS cohorts in comparison to healthy controls. Finally 102 women with early exposure to natalizumab may be too small a number to rule out risk for rare outcomes, and much larger numbers are needed.

As natalizumab interruption has been associated with disease rebound, even during pregnancy\textsuperscript{94} which is typically a remissive period for women with MS,
our study does provide some reassurance to women who inadvertently or intentionally become pregnant while on this drug. As monoclonal antibodies do not typically cross the placenta during organogenesis, the low birth defect rates reported in our study recapitulates this concept, however later transfer in the second and third trimester do occur and one small study associated hematologic abnormalities in 10 out of 13 infants exposed to the drug in the third trimester.69 Given that majority of the exposures occurred just prior to conception and within 13 weeks of gestation, our study is not informative for late pregnancy exposure and women who choose to stay on treatment must practice caution.

Future studies on gestational exposure to natalizumab can elaborate and improve upon our findings by investigating outcomes by matching women on duration of exposure and disease severity. A disease-severity matched comparison group, comprised of women that voluntarily discontinue natalizumab three months prior to pregnancy (as the drug has an estimated mean half-life of 16 ± 4 days) would be ideal. Neonatal and pregnancy outcomes should be assessed in context of disease outcomes during pregnancy and postpartum periods. A longer follow-up duration of the infants exposed, with a focus on immunological abnormalities, specifically in infants exposed beyond the first trimester, would be informative.

In the next two manuscripts we report on the usage of Vitamin D, a supplement that has become popular for its immunomodulatory role in MS patients, and its impact on disease and neonatal outcomes.
In manuscript two we examined disease resurgence—time to first postpartum relapse—in German women with no gestational Vitamin D intake to Canadian women supplementing regularly with Vitamin D. We did not find a significant difference between the two cohorts. This lack of difference in face of the greater and longer duration of exposure to DMDs in the German cohort was a surprising finding, especially as disease severity appeared to be comparable between the two cohorts.

By far the most important decision in the postpartum period in women with MS, who are aware of the risks of disease flare-up, is the decision to breastfeed or not and the length of time to delay treatment re-initiation, in order to extend breastfeeding duration. In women with very active disease wishing to breastfeed but unable to delay treatment, choosing the DMD that is compatible with breastfeeding becomes the main concern. While INFB-1a, and glatiramer acetate appear to have minimal transfer rate into breastmilk, most second line DMDs have not been investigated and are not viable options for breastfeeding moms. As disease state prior to and during pregnancy appear to somewhat predict postpartum disease state, some women and healthcare providers will defer the decision on postpartum treatment type, until after delivery. At which point, depending on disease course and the mother’s wishes on breastfeeding a strategy is employed. Hence in women with RRMS wishing to breastfeed, delaying disease resurgence postpartum is highly desirable and would impact the duration an infant breastfeeds.

As evidence builds on the therapeutic potential of Vitamin D in MS, and given that breastfeeding typically increases the maternal need for Vitamin D
supplementation in order to meet the infant’s demand, \textsuperscript{22} in women with MS, taking Vitamin D may be further justified.

Thus unlike DMDs, Vitamin D may allow women the opportunity to breastfeed for a longer duration, protecting the mother from debilitating disease flare-up while serving the infant’s own vitamin needs.

The major study limitation is the comparison of women belonging to different pregnancy registries in different countries; and while the nature of the disease may not be different between Germany and Canada, differences in the design and administration of the registries, inter-cultural/dietary/lifestyle/racial and genetic factors differences that drive Vitamin D status most certainly question the validity of this comparison. However, as discussed in the manuscript, many biasing factors, such as racial differences, latitude of the countries, season of pregnancy and climate were more similar between the cohorts than anticipated, and since studies have shown supplementation and sunlight to be the most effective way of increasing serum 25(OH)D (with dietary contribution to be minimal), and since only Germans with no Vitamin D intake including prenatals were selected for this study, a marked difference between serum levels of the two cohorts is expected. Of course the lack of serum measurement is another major limitation of our study, and having this information would have certainly helped us understand the lack of difference between the cohorts. Assuming disease severity to be similar between the cohorts – as implied by the lack of major differences in age at disease diagnosis, duration of disease at conception, and disease activity prior to pregnancy between the two cohorts—perhaps the lack of a more conspicuous
protective effect in the Germans using DMDs, may be due to Vitamin D deficiency/insufficiency. We did observe in our sub-analyses a protective effect in the Canadian cohort that initiated DMDs within 2 months of delivery, and although this difference did not reach statistical significance, the synergistic effect of some DMDs in the presence of adequate Vitamin D has been previously reported.\textsuperscript{33} Furthermore the use of prenatals and other supplements may have exposed the Canadians to other vitamins with protective properties. Recent attention has been given to the role of probiotics, B12, Omega 3 and other neuroprotective nutrients in MS.\textsuperscript{98} Future studies should focus on postpartum disease course, in a larger cohort of women within a geographical proximity, while matching for serum calcidiol levels, and taking into account the use of any other supplements and lifestyle behaviors namely exercise and sun exposure. The decision to forgo of treatment for the sake of breastfeeding comes at a significant cost to the mother in the short and long term course of the disease. Impact of a 6-24 month (typical breastfeeding duration) therapy suspension on disease progression has not been investigated, and longitudinal studies are required. The protective effect of exclusive breastfeeding on postpartum disease course, how it contends against DMDs alone, and the added benefit if any, of supplementation with Vitamin D, need to be addressed in future studies. Other outcomes of interest should include frequency and severity of relapses, steroid administration, changes in disability scores, and objective measures of overall maternal wellbeing. All these outcomes contribute significantly to a new mom’s ability to cope with the demands of the newborn and her ability to
breastfeed while optimally managing disease.

In the third manuscript we took a closer look at the dosage of Vitamin D in the Canadian cohort and observed 29 pregnancies with exposures above 4000 IU/day. Nearly all the women in the cohort had been supplementing for several years and despite never measuring serum calcidiol, continued to do so during pregnancy. Studies on gestational Vitamin D and pregnancy have primarily focused on maternal and/or cord blood serum calcidiol levels and the association to specific adverse outcomes, such as pre-eclampsia, gestational diabetes, birth weight, birth length, head size, rates of miscarriage, stillbirths, preterm births, rates of SGA, and occurrence of specific infections in the prenatal and neonatal periods.\(^83,99-104\) In trials where women were supplemented with exogenous Vitamin D, only subjects with established deficient/insufficient status were utilized, and the studies varied substantially from one another in the duration and timing of Vitamin D exposure, the type of Vitamin D (D\(_3\) vs. D\(_2\)), formulation (IV vs. oral), dosing interval (once a month, daily or once a week), as well as the usage of comparison groups (placebo, low dose, or low dose with calcium).\(^83\) Not surprisingly the findings from these studies are ambivalent and inapplicable to our cohort. Given that dosage of Vitamin D and duration of treatment are established factors in predicting serum calcidiol, the consistency in taking a Vitamin D supplement in our cohort from the time of disease diagnosis as part of MS management, means that majority of these women were exposed for several years, to daily doses of Vitamin D at the start of their pregnancies.\(^105\) Thus the likelihood of deficiency is very low and the fetal safety of additional supplementation in
pregnancies of such women is unknown. Here we had an opportunity to compare neonatal outcomes in women that exceed 4000IU/day to women that take lower doses, and we found no increase in adverse pregnancy and neonatal outcomes. For our primary outcome, infant size, we observed no differences in birth weight, adjusted birth weight percentiles and birth length, however we did see significantly larger head circumferences in the high dose group. Upon exploratory analysis the difference in head circumference while still larger in the high dose group, did lose significance after adjusting for gestational age. However as Vitamin D in pregnancy has been shown to play an important role in fetal growth, the observed increase in head size, especially in the context of MS pregnancies (where smaller babies have been reported anecdotally), should be investigated in future studies.\textsuperscript{80} As already discussed in the manuscript, the observed small differences in all parameters of infant size between the high and low dose groups, may render our sample size highly inadequate for capturing a true difference. Furthermore the lack of data on all dietary and lifestyle factors that can impact serum calcidiol (i.e. sunbathing, wintertime tropical vacationing, regular fatty fish consumption, etc.) frays our assumption about lower serum calcidiol levels in the low dose groups. Unfortunately the lack of serum level measurement is a major limitation in this study as well. More recently, a blunted response to Vitamin D supplementation in MS patients has been suggested, and may be due to variants in Vitamin D metabolizing genes.\textsuperscript{106} This suggests that patients with MS may require higher doses of Vitamin D to establish the same serum calcidiol levels as healthy controls, and might be another reason for the lack of observed difference between the high and low dose groups.
The combined impact of disease and pregnancy driven changes on serum calcidiol levels in response to supplementation is critical and must be investigated in future studies. These studies should involve the use of a much larger cohort with maternal serum levels collected in each trimester of pregnancy and postpartum, as well as cord blood levels. We advise that the disease groups be matched for disease severity using objective measures such as disability scores and other clinical parameters. We also recommend including healthy cohorts on the same dosage of Vitamin D in pregnancy. This kind of study will provide novel insight not only on serum response to supplementation by dose and disease status, but will help establish guidelines for women with MS who wish to incorporate this vitamin as part of their disease management and to determine optimal dosages that may be different than general population dose recommendations.

Much research is needed in deciding the “optimal” serum calcidiol levels and the corresponding doses for achieving these levels. Given that many factors impact serum levels, it is likely that individualized doses may be necessary.

**Conclusion**

Many gaps in knowledge related to pregnancy in MS continue to exist. Much has yet to be addressed regarding optimal management of this very dynamic disease during pregnancy and the postpartum year, with significant implications not just for the mother but her newborn. We have investigated two common MS therapies in our pregnant cohorts; natalizumab and Vitamin D. Our results suggest that women with exposure to these therapies may have
normal pregnancy and neonatal outcomes and we highlight the weaknesses of our studies and make recommendations for future studies. Long term follow-up of natalizumab exposed infants beyond the first trimester of pregnancy is needed. We also recommend serum calcidiol monitoring in women with MS and conducting studies that will determine the optimal serum levels and doses required to achieve those levels in this population.

Through our findings we contribute to the foundation for future research to build upon, to determine how to optimize maternal disease management while protecting the fetus and breastfeeding infants of mothers with Multiple Sclerosis.
BIBLIOGRAPHY


15. Bishop M, Rumrill PD. Multiple sclerosis: Etiology, symptoms,


85. Greer FR. 25-hydroxyVitamin D: Functional outcomes in infants and young


99. Wei SQ. Vitamin D and pregnancy outcomes. *Curr Opin Obstet Gynecol.*


## General Information

**CASE #**

**Date(s) of Contact** ____________ **Pregnancy Status**

- [ ] first trimester ____________ Weeks 2nd trimester ______ Weeks third trimester ______ Weeks Breastfeeding ______
- ____________ Month(s) old baby
- Pregnancy Loss
- Planning

Consenting to study participation and follow-ups?  **Yes**  **No**
If not, reason for refusal
DEMOGRAPHICS

Pre-Pregnancy Weight: _________  Current Weight: _________  Height: __

Geographic location

Birthplace

Patient: ________________  Mother: ________________  Father: ________________

Maternal Roots: ______________________  Paternal Roots: ______________________

Residence & Immigration Details

Relationship Status

Currently: _________  Duration of current Status: ________________

Year of diagnosis: _________  Status at diagnosis: _________  Partner at Diagnosis: ________________

Type of impact diagnosis with MS had on the relationship:

Education

Highest Level of Education:

High school  College  University  Post-graduate degree
Field of Study: ________________

Occupation:

Current Job: ______________________  # Hours/week
FAMILY HISTORY

#Sisters (F): ______  #Brothers(M): _______ You are
child #: ___________  Sibling Order & Age difference

Chronic Health conditions in 1st degree relatives? Age/duration of dx.

Mother

Father

Siblings

Is there any family history of birth defects or MS in either of your parents or siblings or 2nd/3rd degree relatives?

Mother

Father

Siblings

OTHER MEDICAL CONDITIONS

<table>
<thead>
<tr>
<th>Chronic Illness + (Diagnosis Date)</th>
<th>Type/Duration of Treatment</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
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</table>
### HISTORY OF RECREATIONAL HABITS

<table>
<thead>
<tr>
<th>Type/Frequency</th>
<th>Age started/stopped</th>
<th>Current status</th>
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</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
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<tr>
<td>Rec. Drugs</td>
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<tr>
<td>Alcohol</td>
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</table>

### MULTIPLE SCLEROSIS DISEASE HISTORY AND DETAILS

Date of official diagnoses by a physician?

**Date:** ________________________________

**MS Category:**

1. Primary Progressive MS  
2. Primary Relapsing MS  
3. Remitting-Relapsing MS  
4. Secondary Progressive MS  
5. Other (Specify)  

**Number of MRI:** __________

**Dates of most Recent MRIs (1,2,3,etc)** ________________________________

**Date of 1st Relapse:** ________________  
Details of 1st Relapse

**Age of hindsight**

**Symptoms:** ___________________________  
Details of hindsight symptoms:

**Total number of attacks since diagnosis:** __________

**Total Lifetime Attacks:** __________

**Total number of attacks confirmed by Neurologist:** __________
## Relapse Details

<table>
<thead>
<tr>
<th>#</th>
<th>Date</th>
<th>Symptoms</th>
<th>Duration</th>
<th>Severity/Impact</th>
<th>Treatments</th>
<th>Triggers?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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</table>

Do Attacks fully resolve?__________________________

Residual Symptoms Type of impact on mobility? .
### Remission Details

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Side</th>
<th>Frequency</th>
<th>Severity/Impact</th>
<th>Treatment</th>
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</thead>
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</tbody>
</table>

Which of your symptoms bother you the most:  

How is your mobility compromised?

What is the order of symptoms (oldest to most recent)  

-  
-  
-
## MS Treatment Details

<table>
<thead>
<tr>
<th>Drug</th>
<th>Start Date</th>
<th>Stop Date</th>
<th>Side Effects</th>
<th># attacks on meds</th>
<th>Discontinuation Reason</th>
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</table>

Additional comments/details on treatments.
**Family Obstetrics History**

**Patient’s Mother’s Pregnancy History**

<table>
<thead>
<tr>
<th>Gx</th>
<th>Outcome</th>
<th>Delivery/Pregnancy complications</th>
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</table>

**Patient’s past pregnancies history**

<table>
<thead>
<tr>
<th>G</th>
<th>P</th>
<th>SA</th>
<th>TA</th>
<th>Ectopic</th>
<th>Molar</th>
<th>Stillbirth</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td></td>
<td></td>
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<td>Ret/Pros?</td>
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<td>G2</td>
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<td>Ret/Pros?</td>
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<td>G3</td>
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<td>Ret/Pros?</td>
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<tr>
<td>G4</td>
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<td>Ret/Pros?</td>
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<td>G5</td>
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<td>Ret/Pros?</td>
</tr>
</tbody>
</table>
Exposures Prior to Conception and During Pregnancy
(Medications, supplements, implants, alcohol, smoking, recreation, etc.)

<table>
<thead>
<tr>
<th>Date Information Collected</th>
<th>Exposure type</th>
<th>Dosing/frequency</th>
<th>Indication</th>
<th>Start-Stop Dates</th>
<th>Notes/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interview 1</td>
<td>Prenatal Brand:</td>
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<tr>
<td>Interview 2</td>
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<tr>
<td>Interview 3</td>
<td></td>
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</tbody>
</table>
Interview Two Intake Form - Pregnancy Interview

MULTIPLE SCLEROSIS CONTROL CASES

Case #

Date of Previous Call: Call Type: 
GA at call: Call 
Current Follow up Date: GA at call 
Type 
PREGNANCY STATUS 
Planned Pregnancy? IVF/IUI/Natural/Pregnancy Aid 

Time to conceive: Current Weight: Weight Gain: 
LMP: EDD: By Dates or 
U/S?

Nausea and Vomiting of Pregnancy

Mild/Moderate/Severe? # Hours of Nausea/day: # Episodes of Vomiting

-----------------------------------------

-----------------------------------------

TESTS AND ULTRASOUNDS

Prenatal screenings Yes/No


Date/GA: ___________ Reason: ___________________________ Finding: 
Date/GA: ___________ Reason: ___________________________ Finding: 
Date/GA: ___________ Reason: ___________________________ Finding: 

Most Recent Ultrasound

Date/GA: ________________(wks/mo) Reason: ___________ Result: ___________
### MS COURSE DURING PREGNANCY

<table>
<thead>
<tr>
<th>Old Symptoms</th>
<th>Frequency &amp; Severity</th>
<th>Severity (Improved, Same, Worsened)</th>
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<table>
<thead>
<tr>
<th>New chronic/recurrent symptoms</th>
<th>Onset</th>
<th>Frequency/Severity</th>
<th>Management/treatment</th>
</tr>
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<tbody>
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</table>

**Chronic MS Symptoms During Remission**

MS course compared to Pre Pregnancy:  Much Better  Better  Same  Worst  Much Worst

MS Course compared to last call:  Much Better  Better  Same  Worst  Much Worst

**Details of Pregnancy Relapse and/or Exacerbations**

<table>
<thead>
<tr>
<th>Date/GA</th>
<th>Symptom(s)</th>
<th>Severity (mild/moderate/severe)</th>
<th>Steroids?</th>
<th>Duration to recover</th>
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</table>

Fully Recovered from Relapse?

Details of Treatment: 

Any Specific Triggers?__________________________
Other Health Conditions (Chronic conditions/pregnancy complications/infections)

<table>
<thead>
<tr>
<th>Conditions/infections</th>
<th>Date of Dx</th>
<th>Treatment</th>
<th>Duration to recover</th>
<th>Current status</th>
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</table>

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## Interview Three-Postpartum Details

### MULTIPLE SCLEROSIS CONTROL CASES

**Date at last call:**

**GA at Last call:**

**Current follow up date:**

**EDD by U/S**

**Maternal Weight ADD**

**Actual Due Date**

**Total Weight Gain in Pregnancy**

**# weeks postpartum?**

**Pregnancy Outcome:**

---

*For Terminations, Stillbirths, Spontaneous abortions, fill out appropriate chart.*

### Live Birth Details

<table>
<thead>
<tr>
<th>Child Order (1st, 2nd, 3rd born?)</th>
<th>Male/ Female</th>
<th>BW (g)</th>
<th>Length</th>
<th>Head C.</th>
<th>Delivery mode</th>
<th>APGAR Scores</th>
<th>Epidural</th>
<th>Forceps</th>
<th>Vaccum</th>
<th>Labour Length (hr)</th>
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</table>

**Delivery Complications?**

---

*For Neonatal Health & Birth defects fill out appropriate chart*

<table>
<thead>
<tr>
<th>Condition(s)</th>
<th>Diagnosis</th>
<th>Severity</th>
<th>Treatment</th>
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</thead>
<tbody>
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</table>

**Birth Defect**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Severity</th>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
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</table>
Breastfeeding Details at Interview

<table>
<thead>
<tr>
<th>BF Start</th>
<th>Exclusive BF?</th>
<th>Duration for EBF</th>
<th>Breastfeeding Stop date</th>
<th>Reason to Discontinue</th>
</tr>
</thead>
<tbody>
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</table>

Feeding Problems? MS impact on breastfeeding?

---

Multiple Sclerosis Related Health

<table>
<thead>
<tr>
<th>Past Chronic MS Symptoms</th>
<th>Frequency &amp; Severity</th>
<th>Treatments/Management</th>
<th>Comparison to Pregnancy</th>
<th>Comparison to Pre-preg</th>
</tr>
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</table>

New Chronic Symptoms

DMD Start Date

Impact on BF:

---

Relapses/ Attacks in late pregnancy and PP

<table>
<thead>
<tr>
<th>Relapses since Delivery</th>
<th>Symptoms and Severity</th>
<th>Steroids form and duration?</th>
<th>Fully recover?</th>
<th>Duration to recover</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

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## Other Health Conditions

<table>
<thead>
<tr>
<th>Conditions/infections</th>
<th>Episodes</th>
<th>Date of Dx</th>
<th>Treatment</th>
<th>Duration to recover</th>
<th>Current status</th>
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</tbody>
</table>
Interview Four-Twelve Month Follow-Up

MULTIPLE SCLEROSIS CONTROL CASES

Today’s Date: Child’s Age months

INFANT DEVELOPMENTAL MILESTONES

Number of weeks premature: ____________
Adjustment for prematurity: _

Age Reaching Milestones

Rolled over: Mon: Not yet
Attended to and reached for objects: Mon: Not yet
Sat up without support: Mon: Not yet
Turned head to locate a voice/noise: Mon: Not yet
Said “mama” or “dada”: Mon: Not yet
Stood alone: Mon: Not yet

INFANTS MOST RECENT STATUS

Date at last checkup:
Height cm/inches Weight kg/lbs
Head Circumference cm/In. Breastfeeding No/Yes #
BF/Day Age when Exclusive BF stopped:

Reason to Stop Breastfeeding:
Age when solids started: months
Infant’s Health Conditions

<table>
<thead>
<tr>
<th>Conditions/infections</th>
<th>Episodes</th>
<th>Date of Dx</th>
<th>Treatment</th>
<th>Duration to recover</th>
<th>Current status</th>
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</table>

MATERNAL HEALTH AT 1 YEAR (Update Drugs and Exposure Sheet)

<table>
<thead>
<tr>
<th>Chronic MS Symptoms (new?)+ Onset date</th>
<th>Frequency &amp; Severity</th>
<th>Treatments/Management</th>
<th>Comparison to Pregnancy</th>
<th>Comparison to Pre-preg</th>
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</table>

MS Relapses Since Delivery

<table>
<thead>
<tr>
<th>R-Date(s)</th>
<th>Symptoms + Severity</th>
<th>Treatments</th>
<th>Duration to recover</th>
<th>Fully Resolve</th>
<th>Triggers?</th>
</tr>
</thead>
<tbody>
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Most Recent MRI? Results?
Decision to Start DMD? DMD
Type: Start Date: Impact on breastfeeding?

Mother’s Other Health Conditions

<table>
<thead>
<tr>
<th>Conditions/infections</th>
<th>Episodes</th>
<th>Date of Dx</th>
<th>Treatment</th>
<th>Duration to recover</th>
<th>Current status</th>
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## Appendix 3-Expanded Disability Status Scale Grading System

Table 1: Kurtzke’s Expanded Disability Status Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Function Systems involved and degree of disability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>Normal neurological exam (a score of zero in all functional systems)</td>
</tr>
<tr>
<td>1.0</td>
<td>No disability, minimal signs in one FS</td>
</tr>
<tr>
<td>1.5</td>
<td>No disability, minimal signs in more than one FS</td>
</tr>
<tr>
<td>2.0</td>
<td>Minimal disability in one FS (one FS grade 2, others 0-1)</td>
</tr>
<tr>
<td>2.5</td>
<td>Minimal disability in two FS (two FS grade 2, others 0-1)</td>
</tr>
<tr>
<td>3.0</td>
<td>Full ambulatory. Moderate disability in one FS (one FS grade 3, others 0-1), or mild disability in three or four FS (three to four FS with grade 2, others 0-1)</td>
</tr>
<tr>
<td>3.5</td>
<td>Full ambulatory but with moderate disability in one FS (one grade 3, and one or two FS with grade 2, or two FS with grade 3, or five FS with grade 2, others 0-1)</td>
</tr>
<tr>
<td>4.0</td>
<td>Full ambulatory without aid; can walk 500 meter without aid or rest. Self-sufficient and up and about some 12 hours a day despite relatively severe disability consisting of one FS with grad 4 (others 0-1), or combination of lesser grades in FS exceeding previous steps</td>
</tr>
<tr>
<td>4.5</td>
<td>Fully ambulatory without aid; able to walk without rest or aid 300 meters. Up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability, usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps.</td>
</tr>
<tr>
<td>5.0</td>
<td>Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (eg, to work full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0.)</td>
</tr>
<tr>
<td>5.5</td>
<td>Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding those for step 4.0.)</td>
</tr>
<tr>
<td>6.0</td>
<td>Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)</td>
</tr>
<tr>
<td>6.5</td>
<td>Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)</td>
</tr>
<tr>
<td>7.0</td>
<td>Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheel chair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone)</td>
</tr>
<tr>
<td>7.5</td>
<td>Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+)</td>
</tr>
<tr>
<td>8.0</td>
<td>Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)</td>
</tr>
<tr>
<td>8.5</td>
<td>Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally 4 + in several systems.)</td>
</tr>
<tr>
<td>9.0</td>
<td>Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4 +)</td>
</tr>
<tr>
<td>9.5</td>
<td>Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4 +)</td>
</tr>
<tr>
<td>10.0</td>
<td>Death due to MS</td>
</tr>
</tbody>
</table>

*Adapted from Neurology (Cleveland) 1983;33:1444-52