Cord Blood Vitamin D Status and Neonatal Outcomes in a Birth Cohort in Quebec

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
November 2013

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Abstract

Vitamin D status is assessed with circulating 25-hydroxyvitamin D [25(OH)D]. As some evidence suggests that low vitamin D status adversely affects neonatal health, this project aimed to determine the association between cord blood 25(OH)D levels and preterm birth (PTB; <37 weeks gestation), low birthweight (LBW; <2500 grams) and small for gestational age (SGA; <10th percentile) and to examine the relationship between maternal 25(OH)D levels during the first trimester of pregnancy and fetal 25(OH)D levels at birth in a Canadian population.

This nested case-control study used serums, questionnaires and chart reviews collected in Quebec City. Compared to 25(OH)D concentrations ≥75 nmol/L, concentrations 37.5-<75, 50-<75, and <75 nmol/L were associated with lower odds of LBW, PTB and an adverse neonatal composite outcome, and PTB as well as LBW, respectively. Maternal and neonatal 25(OH)D were correlated ($r=0.23$, $p<0.01$; adjusted $r=0.46$, $p<0.01$). This study contributes to evidence for identifying further policy and research directions.

List of Abbreviations and Symbols Used

<: less than

>: greater than

≤: less than or equal to

≥: greater than or equal to

±: plus or minus

25(OH)D : 25-hydroxyvitamin D

aOR : adjusted odds ratio

BMI : body mass index

CEGEP : Collège d'enseignement général et professionnel

CHD : coronary heart disease

CI : confidence interval

CIHR: Canadian Institutes for Health Research

cm : centimetre

DEQAS : Vitamin D External Quality Assessment Scheme

et al.: et alii (and others)

g: grams

kg: kilograms

HADS: Hospital Anxiety and Depression Scale

IQR: interquartile range

IUGR: intrauterine growth restriction

LBW: low birthweight

LMP: last menstrual period

m: metres

MCMC: Markov Chain Monte Carlo

MI: multiple imputation

n: number

nmol/L: nanomoles per litre

OR: odds ratio

PTB: preterm birth

VDR: vitamin D receptors

vs.: versus

r: correlation coefficient

RCT: randomized controlled trial

Ref.: reference

SD: standard deviation

SES: socioeconomic status

SGA: small for gestational age

Acknowledgements

I would like to thank my thesis committee members for their patience and dedication throughout this project. Thank you to my supervisors Dr. Linda Dodds and Dr. Christy Woolcott for the countless hours that you have spent guiding me through this process. You helped me understand issues ranging from coding details to the bigger picture my work. Thank you for sharing your endless enthusiasm and your inquisitive natures with me. Thank you to my committee member Dr. Donald Langille for your continued support and kindness. You have been a wonderful mentor and I am deeply grateful for the contribution that you have made to this team.

Thank you to my office-mates Anne Spencer, Mark Staples and Pamela Zimmer. The many questions and answers that you shared in the office have contributed to the quality of work presented in this document. You have been a pleasure to work beside over the past year. I would like to thank my friends and family for their love and support through the ups and downs of this process. I would especially like to thank my mum, Ann Wilton, for cheering me on and inspiring me to strive for excellence in work and balance in life.

This project would not have been possible without the office space provided by the IWK Health Centre and the funding provided by CIHR.

CHAPTER 1: INTRODUCTION

1.1 Background and Study Rationale

Vitamin D is a fat-soluble secosteroid that can be derived endogenously or ingested (1,2). When ultraviolet-B (UV-B) radiation from the sun hits the skin, it activates pro-vitamin D_3, which is converted into cholecalciferol (vitamin D_3) (3-5). Additionally, vitamin D can be consumed in the form of cholecalciferol (vitamin D_3) as well as ergocalciferol (vitamin D_2) (3,4). Vitamin D_3 and vitamin D_2 are then transformed into 25-hydroxyvitamin D [25(OH)D] in the liver (6). This form, 25(OH)D, with a reported half-life of approximately two months (7,8), is the main circulating form of vitamin D and the best measurement of overall vitamin D status (9,10).

Vitamin D targets vitamin D receptors (VDRs) to serve both 'classic,' bone-related functions, as well as 'non-classic,' beyond-bone-related functions (11). It is involved in the uptake and degradation of calcium and phosphorous in bones from serum (11,12). Low vitamin D has been implicated in the occurrence of rickets, a bone disease that leads to soft or weak bones, for nearly a century (1). Vitamin D has also been shown to play a role in cell differentiation, cell growth, metabolism and immunity (11). There is growing interest in and evidence on the role of vitamin D associated with these non-classic functions (13).

Vitamin D insufficiency is common in Northern climates, with 67% of Canadian males and 62% of Canadian females having levels below the recommended level of 25(OH)D $\geq$75 nanomoles per litre (nmol/L) (14). There are particularly low levels of

vitamin D during the winter in Northern climates (15,16). The increased zenith angle of the sun in the winter decreases UV-B radiation and pre-vitamin D_3 production as a result (16). Pre-vitamin D_3 synthesis has been shown to be negligible after 3 hours of skin exposure to sunlight from November to February in Boston (latitude 42°N) and from October to March in Edmonton (52°N) (16). Quebec City's latitude falls between these two locations.

Of Canadian women of reproductive age, 63.7% have been shown to have vitamin D status below the optimal 75 nmol/L (14). Vitamin D during pregnancy is particularly concerning because of the dependence of the growing fetus on its mother to be vitamin D sufficient (≥75 nmol/L) (17) and because levels of vitamin D tend to be lower in pregnant women than in equivalent non-pregnant women (18).

There is controversy about the optimal vitamin D status for the fetus, vitamin D's effects on various outcomes, and the relationship between maternal and fetal vitamin D (4,5,19-32). The role of vitamin D status during pregnancy and at birth on adverse outcomes beyond bone-related ones remains largely overlooked (10,33). The outcomes of interest for this study, preterm birth [PTB; less than (<) 37 weeks gestation], low birthweight [LBW; <2500 grams (g)], and small for gestational age (SGA; <10^{th} percentile), have rarely been examined in direct relation to cord blood vitamin D status. The 'clinical upshot' to the current study is that if vitamin D insufficiency is found to be associated with increased risk of PTB, LBW and/or SGA compared to vitamin D sufficiency, this will inform the need to conduct randomized controlled trials to improve fetal health with maternal vitamin D supplementation during pregnancy.

The major call for the current study comes from the lack of research directly measuring the relationship between cord blood vitamin D status and PTB, LBW and SGA infants. There remains considerable debate over the serum concentrations of 25(OH)D associated with optimal health, and cut-off points have not been developed by a scientific consensus process (34).

Novel data available in Quebec City (latitude 47°N) provides a unique opportunity to answer some of the scientific questions about vitamin D during pregnancy. The primary reason for choosing Quebec City to investigate vitamin D in cord blood and associated outcomes is that investigators at Laval University have stored blood samples from a birth cohort in which approximately 8,000 women were recruited from Quebec City, along with corresponding questionnaires and medical chart reviews. Investigators affiliated with Dalhousie University, Laval University and McGill University are collaborating on this project.

1.2 Study Objectives

The overall goal of this project is to evaluate the association between vitamin D status in umbilical cord serums with adverse neonatal outcomes in order to contribute to the evidence necessary for identifying further research and policy directions. This study aims to contribute to a body of knowledge for evidence-based vitamin D supplementation recommendations during pregnancy for mothers, particularly for populations living at Northern latitudes.

i) The primary objective is to determine whether vitamin D status in cord blood is associated with PTB, LBW and SGA.

ii) The secondary objective is to examine the relationship between maternal vitamin D status during the first trimester of pregnancy and fetal vitamin D status at birth.

CHAPTER TWO: REVIEW OF LITERATURE

2.1 Factors Affecting Vitamin D Status

Vitamin D synthesis and intake are determined by sun exposure, season, and consumption of fortified food and supplements. Vitamin D status is further modified by body-mass-index (BMI), age and genetics. Vitamin D intake from food as well as prenatal vitamins and mineral supplements have been significantly correlated with maternal and cord serum 25(OH)D concentrations (35). Fortified foods are the major nutritional source of vitamin D. Vitamin D is found in fortified milk drinks, butter, margarine, fish, eggs and cheese products (36). In Canada, cow's milk and margarine are required to be fortified (34). Some orange juices that are calcium-fortified are allowed to be fortified with vitamin D, as are drinks such as soy beverages and goat's milk (34). Fatty fish and egg yolks serve as the only natural sources of vitamin D in Canada (34). Vitamin D status has been shown to increase with increasing sun exposure (37). Dror and others (et al.) found a 1-month lag from seasons based on the solstices and equinoxes, with both maternal and cord serums highest in late summer/ early fall and lowest in late winter/ early spring (35). The impact of season on 25(OH)D status can be attributed to the amount of skin that is exposed to the sun on a regular basis (35) as well as the amount of UV-B radiation that reaches the skin (16).

Having a BMI of ≥25 kilograms per metre squared (kg/m^2) has been shown to be an independent predictor for vitamin D deficiency (35,38). The proportion of adults with 25(OH)D <75 nmol/L increased with age in an Australian population-based study (38). Older people are at a higher risk of low vitamin D status because of a decline in the

efficiency of vitamin D synthesis and lower renal conversion to its active form (39). Ethnicity has been shown to be a factor in vitamin D status (38). The highest rates of deficiency and insufficiency have been found in non-Caucasians (38). Vitamin D insufficiency prevalence has been highest in African-American mothers and their infants (35).

Recent research has examined the genetic influences on vitamin D status. Wang et al. (40) conducted an extensive study involving 33,996 participants of European descent from 15 cohorts. The researchers were able to establish a role for common genetic variants in the regulation of 25(OH)D concentrations. However, these variants were estimated to be responsible for only 1-4% of 25(OH)D variability (40).

2.2 Vitamin D in Pregnant Mothers and Neonates (Canadian Focus)

Cut-off points for classifications of vitamin D sufficiency/ optimal levels and potentially harmful levels of vitamin D are based primarily on bone-related outcomes (34). The Canadian Paediatric Society's guidelines for children and pregnant women uses cut-offs of ≥ 75 nmol/L for optimal, greater than (>) 225 for potential adverse effects, and >500 for potentially toxic (41). The upper limit is in place in order to try to minimize adverse effects of too much vitamin D, including the accumulation of calcium salts in the kidney and other soft tissues (34). It is possible that the current definition of vitamin D deficiency and insufficiency based on bone-related disease is not appropriate because other health outcomes need to be taken into account (33). However, the current evidence on calcium and vitamin D and its association to cancer, cardiovascular disease, diabetes,

and immunity has not been of high enough quality to support a cause-and-effect relationship (34). This illustrates the need for better research in these areas.

Low levels of vitamin D are common in Northern populations regardless of pregnancy status, but are particularly concerning among pregnant women. The Canadian Health Measures Survey collected blood samples in 2007-2009 from a nationally representative sample of 5,307 individuals aged 6-79 years (14). Of particular interest to this study, women of the dominant child-bearing ages (20-39 years) had a mean 25(OH)D concentration of 69.5 nmol/L [95% confidence interval (CI) 65.8-73.2] (14). Of this population only 36.3% had levels above 75 nmol/L (14). Pregnant women's vitamin D statuses are on average lower than equivalent non-pregnant women's statuses (18). This difference in vitamin D status between pregnant and non-pregnant women is hypothesized to be partly caused by the fetal demands for vitamin D (18).

A study by Holmes et al. included women living 54-55°N of the equator and found that over 95% of pregnant women were classified as having insufficient vitamin D at three different time-points using a cut-off of <80 nmol/L, regardless of whether they reported supplement use or not (18). A study examining pregnant women in Newfoundland and Labrador found mean serum 25-(OH)D concentrations of 52.1 nmol/L in the winter and 68.6 nmol/L in the summer (15). Of this population 95.6% had 25(OH)D concentrations <75 nmol/L in the winter and 65.7% had 25(OH)D concentrations below this level in the summer (15).

2.3 General Prevalence and Risk Factors for, and Consequences of Preterm Birth, Low Birthweight and Small for Gestational Age

2.3.1 Preterm Birth

Prevalence and Risk Factors

In 2004, 8.4/ 100 live births in Canada were preterm (born before 37 weeks gestation) (42). It is important to keep in mind some of the data limitations in this area. Using the last menstrual period (LMP) for dating the start of pregnancy can be inaccurate because of inaccurate reporting by mothers, mistaken bleeding early on in the pregnancy as a normal period, irregular menstrual cycles, or unnoticed pregnancy losses (42). Fortunately, in an effort to address these problems, ultrasound confirmation of gestational age is commonly used in Canada (42).

The etiology of PTB is multifaceted. Potential risk factors for PTB include body-mass-index (BMI), smoking, physical activity, maternal socioeconomic status (SES- in general, marital status, education, occupation, and family income), ethnicity, emotional distress, season of measurement, alcohol, and drug use. A recent systematic review of maternal BMI and PTB (43) suggested that there is not a strong association with obesity in general and PTB. In examining 20 cohort studies, with a total of nearly 2 million participants, the meta-analysis revealed a pooled adjusted odds ratio (OR) for obese mothers as a whole to be 0.89 [95% confidence interval (CI) 0.7-1.01] compared to women with a normal BMI (classified as 20-24.9kg/m^2 in this analysis) (43). Among overweight (BMI 25-29.9) and obese class I (BMI 30-34.9), the pooled adjusted ORs were 0.93 (95% CI 0.86-1.01) and 1.08 (95% CI 0.95-1.23) respectively (43). There were

statistically significant differences among the obese class II and class III versus normal weight mothers' risk of delivering preterm, with the adjusted OR for women with a BMI of 35-39.9 at 1.33 (95% CI 1.12-1.57) and an adjusted OR for women with a BMI ≥40 of 1.83 (95% CI 1.62-2.09) (43). Hence BMI at these levels may still be worth examining as potential covariates for PTB.

Two randomized controlled trials (RCTs) examining the impact of exercise interventions on pregnant women and their risk of adverse neonatal outcomes found no statistically significant differences in risk of PTB between their exercise and control groups (44,45). The first study (44) included nulliparous pregnant women who did not partake in formal exercise exceeding 60 minutes per week, nor brisk walking for more than 120 minutes per week in the 6 months prior to the study, and who were able to attend weekly exercises. Members of the exercise group engaged in two aerobic dance classes per week for a minimum of 12 weeks (44). Participants in this group were also told to incorporate 30 minutes of moderate physical activity into non-aerobic-class weekdays (44). This study found no statistical difference in the length of gestation between the exercise and control groups (44). Nascimento et al. included expectant mothers with pre-gestational BMIs that classified them as overweight (26-29.9 kg/m^2 in this study) and obese (≥30 kg/m^2), undergoing singleton pregnancy, with a previously sedentary lifestyle (45). This study included 80 women and assigned one arm to an exercise program and the other to a control group. The exercise program included weekly classes of light to moderate exercise as well as home exercise counseling to be done five times per week (45). The control group was not given physical activity counseling (45). This study found no significant differences for gestational age at birth among the

offspring of the participants (45). Both of these studies prompt the investigation of how exercise influences outcomes among inactive, overweight or obese pregnant women. In a less truncated sample exercise may have a clearer impact.

Socioeconomic status (SES) is a measurement of one's social and financial standing within the larger group. It is composed of various elements including marital status, education, and occupation. Marital status is a common factor associated with SES (46). This status may seem unimportant in the context of Quebec, where 29% of families were common-law in 2006, representing 44% of all common-law couple families in the country that year (47) and cohabitation does not necessarily carry the same socioeconomic differences versus marriage seen in the rest of Canada (48). However, Auger et al. (49) found percentages of PTB to increase based on lower marital status in the Quebec context. Babies born to married mothers were preterm 5.7% of the time, those born to mothers cohabiting with their partners 6.4% of the time, and those born to single mothers were preterm 8.9% of the time (49).

The Quebec study by Auger et al. found that rates of PTB increased with decreased educational levels (49). Maternal education was categorized as no high school diploma, high school diploma, some post-secondary, some university or more, or unknown (49). This study is of particular interest because it was conducted throughout Quebec, the Canadian province in which the current study's data was collected (49). In a recent systematic review, Blumenshine et al. (50) examined the relationship between SES and various measures related to PTB: continuous gestational age, PTB, very PTB, and preterm premature rupture of membranes. The majority of the studies included in this

review that looked at SES and PTB found significant associations between education and occupational status with PTB in the whole sample or at least in a subgroup (50).

Race and ethnicity have been shown to be predictors of PTB rates. This may have both biological and sociological components. Being of African descent compared to Caucasian, has been identified as a risk for PTB (51-53). One possible explanation for this is linked to the fetal inflammatory response system (54). Maternal immune response variations may play a role in the pathophysiology of spontaneous preterm labour and PTB (53). In a recent systematic review, Schaaf et al. pooled the results of 30 studies to calculate a pooled OR of 2.0 (95% CI 1.8-2.2) for Blacks compared to Whites (55). Being Black was independently associated with PTB after controlling for established confounders. There was no significant association found between being Asian or Hispanic and increased risk of delivering preterm in this study. It is worth noting that 80% of the studies included were conducted in the United States where racial discrimination may play a cumulative toll on Blacks. Dominguez et al. suggests that the higher rates of adverse neonatal outcomes among Blacks in the United States are due to the many consequences of racial oppression, such as lower financial earnings even with the same level of education, and increased environmental stress induced by racism (56).

Emotional distress has also been examined in relation to PTB. In a prospective cohort study involving 1,962 women and a psychosocial questionnaire with several standardized instruments found that women in the highest stress quartile had the highest risk of PTB (57). However, for women in the two middle quartiles of stress there was no increased risk (57). The study subdivided types of PTB and found that women with

higher pregnancy-related anxiety were at higher risk of spontaneous PTB than of medically indicated PTB (57).

There is evidence of seasonal variation in gestational age at birth (58). In Japan, where the rainy season begins as early as May, and goes into mid-July, followed by typhoon season from August to October (59), the longest mean gestational periods have been shown in October, with the shortest in winter (58).

Alcohol consumption during pregnancy has been shown to be associated with PTB in a recent review (60). This review identified a study that found three times the likelihood of PTB after fetal exposure to more than 20 units of alcohol per week. There have been mixed findings for the relationship between low levels (1-2 drinks per occasion or less than 70 g per week), but at least one study has found a dose-response for alcohol and risk of PTB starting with only 1-2 drinks per week (60).

The use of illicit drugs during pregnancy can have harmful consequences for both the mother and her offspring (61). Although the prevalence of use decreases during pregnancy, it still persists among some users (62). Marijuana is the most commonly used illicit drug during pregnancy (61,62). A self-report measure revealed a prevalence rate of 2.5% for this substance during pregnancy (62). Stimulants such as ecstasy and opioids such as heroine have also been reported (61-63). A study conducted in New South Wales, Australia, found increased odds of having a preterm delivery with the use of illicit drugs among opioids, marijuana, and stimulants (61). The OR based on opioid use was the most severe, with an adjusted OR (aOR) of 3.9 (95% CI 2.7-3.5), followed by marijuana with an aOR of 2.2 (95% CI 1.9-2.5) and then stimulant use with an aOR of 1.5 (95% CI 1.1-

2.0) (61). Cocaine use in particular has been shown to increase the odds of PTB in a recent systematic review and meta-analyses, with an OR of 3.38 (95% CI 2.72-4.21) (63). Furthermore, this systematic review and meta-analysis found that use of cocaine during pregnancy decreases the gestational period by an average of 1.47 weeks (95% CI 0.98 - 1.97 weeks shorter) (63).

Consequences/ Impact of Preterm Birth

The negative consequences of PTB are far ranging and long lasting. PTB is associated with more neonatal and infant mortality than any other major risk factor in industrialized countries (64,65). Infants born preterm also have more difficulties adapting outside of the womb early on as well as problems with their nervous systems and their behaviour throughout their lives compared to those born at term (66). Even mild to moderate PTB (32-<37 weeks gestation) are a matter of concern. Relative risks for infant death from all causes among singletons born at 32-33 gestational weeks relative to singletons born at 37 weeks or later were 15.2 (95% CI 13.2-17.5) in Canada in 1992-1994 (67). For those born at 34-36 weeks the relative risk for infant death was 4.5 (95% CI 3.9-5.2) (67). With such severe consequences of PTB it is worth assessing the impact of modifiable risk factors.

Vitamin D and Preterm Birth (Objective 1a)

One proposed mechanism for vitamin D's possible influence on PTB is through the vitamin's impact on innate immune responses (68,69). Vitamin D deficiency can impair innate immune function *in vitro* by hindering immune cells' ability to respond to an infection (69). In a multi-centred European birth cohort a positive association between

maternal vitamin D supplementation during pregnancy and levels of two inhibitory receptors were found in cord blood (68). Impaired immune function based on vitamin D status could potentially be related to PTB because of its negative health impact on the growing fetus (68).

The relationship between vitamin D and PTB has been examined using RCTs with vitamin D supplementation as well as observational studies looking at maternal serum and cord blood vitamin D status. In a double-blind RCT involving 350 women receiving vitamin D supplementation beginning at 12 to 16 weeks of pregnancy, Hollis et al. found no difference in gestational age at delivery among the three trial groups (19). The group that received 400 IU per day until delivery gave birth to infants with a mean gestational age of 38.6 plus or minus (±) 2.2 weeks, the group that received 2,000 IU per day had a mean gestational age of 38.8 ± 1.8, and the group that received 4,000 IU per day had a mean gestational age of 39.1 ± 1.8, p=0.17 (19). The group given 4,000 IU per day had the highest mean level of maternal 25(OH)D one month prior to delivery and at delivery compared to both of the other groups in the study, as did their babies at delivery (19). Mothers in the 400 IU, 2,000 IU and 4,000 IU groups had 25(OH)D concentrations of 79.4 ± 34.3, 105.4 ± 35.7 and 118.5 ± 34.9 one month before delivery, and concentrations of 78.9 ± 36.5, 98.3 ± 34.2, and 111.0 ± 40.4 at delivery, respectively. Infants paired with mothers in the 400-IU, 2,000-IU, and 4,000-IU groups had 25(OH)D concentrations of 45.5 ± 25.3 nmol/L, 57.0 ± 24.5 nmol/L and 66.3 ± 25.8 nmol/L at birth, respectively (70). The clustering of all of the maternal levels above 75 nmol/L, and all of the fetal levels well below this cut-off could have impacted why the investigators

did not find a statistically significant difference in the risk of PTB. No additional adverse events were reported in the highest supplemented group.

This study had several strengths. By excluding women >16 weeks gestation from enrolment they were able to look at the effect of supplementation in a more homogenous and long-term way compared to a more flexible or later starting point. They included several relevant socio-demographic measures in their analyses (maternal age at enrolment, self-defined race, insurance status, educational status, and occupation and employment outside the home). Also, they had a multi-ethnic study including blacks, Hispanics and whites, who they balanced in the study arms, thus extending the generalizability of their findings (19). In a different RCT in London England with 179 participants looking at supplementation using either a one-time oral dose of 200 000 IU or a daily dose of 800 IU from 27 weeks gestation until delivery, Yu et al. (20) found no significant difference in gestational age at delivery in either supplemented group compared to no treatment. This occurred despite the participants in the supplemented group having higher vitamin D status than the no treatment group with a median of 34 nmol/L in the one-time treatment group [interquartile range (IQR) 30-46 nmol/L] and 42 nmol/L in the daily dose group (IQR 31-76 nmol/L) compared to a median of 27 nmol/L for the no treatment group (IQR 27-39), $p<0.0001$ (20). The lack of statistical significance between supplementation and gestational age at delivery may be explained by the finding that all of the 25(OH)D concentrations remained low.

Perez-Ferre et al. conducted a prospective cohort study in Madrid, Spain, comprising 266 expectant mothers at 24-28 weeks gestation and their newborns upon delivery (21). Using a cut-off of <50 nmol/L to define vitamin D deficiency, this study

found vitamin D deficiency to increase the risk of delivering prematurely with an OR of 3.31 (95% CI 1.52-7.19, p<0.002) compared to insufficiency and sufficiency (grouped together) (21). The median 25(OH)D for this entire study population was 47.25 nmol/L. This study also found that using a cut-point of 35 nmol/L optimized the trade-off between sensitivity and specificity for PTB, with sensitivity at 66.7% and specificity at 71.0% for this cut-off. This study was well-conducted. The investigators collected information on demographics, obstetric and family history, and lifestyle before and during pregnancy, dietary intake of vitamin D and daily sun exposure. The study also assessed the assay that they used to measure vitamin D using the Vitamin D External Quality Assessment Scheme (DEQAS). Overall this study was useful in its examination of the relationship between maternal vitamin D and risk of PTB, particularly because of its identification of the most useful cut-point for this relationship.

Thorp et al. conducted a nested case-control study within an RCT of women who had undergone at least one previous spontaneous singleton PTB (22). For the purpose of the randomized controlled trial, women at 16-<22 weeks gestation were randomly assigned to take 1200 mg of eicosapentaenoic acid along with 800 mg of decosahexaenoic acid (434 women) or to take matching placebos (418 women). Everyone in the study received 17α-hydroxyprogesteronecaproate injections each week. No association was found between low vitamin D status at 16-<22 weeks and recurrent PTB. When the lowest quartile was compared to the highest, the odds ratio was 1.33 (95% CI 0.48-3.70), and when <50 nmol/L was compared to ≥50 nmol/L the odds ratio was 0.80 (95% CI 0.38-1.69). There was also no association found with 25(OH)D concentrations

in serum taken at 25-28 weeks and PTB, nor with vitamin D and very early PTB (<32 weeks).

Baker et al. conducted a nested case-control through the University of North Carolina-Chapel Hill on women who were originally recruited at 11-14 weeks gestation (23). Forty cases of spontaneous PTB between 23-<35 weeks gestation were matched by race/ethnicity with 120 controls. Deliveries between 35-<37 weeks gestation were excluded in order to avoid potentially misclassified PTB based on incorrect gestational dating. The authors found no association between vitamin D deficiency (<50 nmol/L) during the first trimester and spontaneous PTB compared to vitamin D sufficiency (≥75 nmol/L). The unadjusted odds ratio comparing first trimester vitamin D <50nmol/L versus ≥75 nmol/L was 1.14 (95% CI 0.31-4.26), and the adjusted odds ratio was 0.82 (95% 0.19-3.57).

Hossain et al. conducted a prospective cohort involving 75 mothers and their offspring in Karachi, Pakistan (5). This study included maternal and cord blood at delivery, and a cut-off point of 75 nmol/L for vitamin D sufficiency (5). The investigators in this study found higher maternal and cord blood vitamin D status to be associated with *shorter* gestational periods (r=-0.33, p=0.003) (5).

All of these studies contain limitations. For the large RCT conducted by Hollis (19), the primary objective was to examine the impact of supplementation on vitamin D status, not the impact of vitamin D status on neonatal outcomes (19). The power calculation was based on the ability to detect an increase of 25 nmol/L in vitamin D rather than to be able to detect differences in neonatal outcomes based on vitamin D

status. Additionally, women who had 25(OH)D concentrations between 100 and 150 nmol/L at baseline were not randomized to the highest dosage group, and women with levels above 150 nmol/L were only given the lowest dosage, which could have limited the internal validity of the findings because these subsets were systematically excluded from proper randomization (19). Unfortunately, in the other RCT conducted by Yu, even with supplementation, vitamin D sufficiency was only achieved by 30% of participants in this study (20). Moreover, this study was not blinded and there was no placebo provided for the 'no treatment' group (20).

The major limitations of the study by Perez-Ferre were that the population was limited to women 29-36 years old, which limits the reach of the study's applicability to younger and older mothers (21). The major limitation of the nested case-control study within an RCT of women who had already experienced at least one spontaneous singleton PTB was its high-risk population (22). Although the authors did control for maternal age, smoking, BMI, race/ethnicity, study centre, number of previous PTB, season of measurement, and treatment group, in a population with a greater risk of delivering preterm, there could have been genetic and environmental factors that were not properly accounted for.

The major weaknesses of the study by Baker (23) arise from a lack of power due to the high prevalence of vitamin D sufficiency in this study population. Among study participants, 73% had vitamin D status ≥75 nmol/L, with a median level of 89 nmol/L. The power calculation required a rate of 25% of vitamin D deficiency in the control group, and double that among the cases for 80% power with an α of 0.05. Only 7.5% of the cases had levels below the <50 nmol/L cut-off for deficiency. In addition to power

issues, the sample population in this study was not representative of the entire population as 88% of the participants were privately insured, suggesting a higher income bracket. Finally, considering these cases came from an original cohort of 4225, the number of spontaneous PTB was low, again suggesting that this population was unusually healthy.

The Hossain study (5) also has limitations that are worth taking into account. The study only involved 75 participants. Of these, almost 90% were classified as vitamin D deficient with the cut-off of <75 nmol/L (5). This limited variability in the exposure increased the influence of a few outliers on the perceived trend. Moreover, the cultural practices of women covering their arms, hands and heads as well as the latitude of this Pakistani population decrease the applicability of this study to the Canadian population as a whole.

Although the current study will not address the challenges specific to RCTs, it will fill the gaps left in the literature in the following ways. This study will be more generalizable than the studies by Thorp (22) and Baker (23) since mothers were not excluded on the basis of their age and because Quebec has universal healthcare, hence the population included did not face the same limited population challenges based on the SES measure of private health care as the Baker study (22,23). Moreover, higher rates of vitamin insufficiency are anticipated in the Quebec population (14) than the Baker study (23) based on national rates; therefore the same power issues should not be encountered. The current study will be more generalizable to the Canadian population and populations with similar latitudes and cultural practices than the study conducted by Hossain et al. (5).

2.3.2 Low Birthweight

Prevalence and Risk Factors

The Canadian LBW (<2500 grams) rate was 6/100 live births in 2004 (42). LBW is influenced by both PTB and SGA classifications. While there was an increase in PTBs from the mid-1990s to mid-2000s in Canada, there was a decrease in the number of SGA births, with the LBW rates remaining stable as a result (42). This stability may explain why LBW has received less attention than its counterparts (42).

The potential risk factors for LBW include BMI, smoking, physical activity, maternal socioeconomic status (in general, marital status, education, occupation, and family income), ethnicity, emotional distress, season of measurement, alcohol, and drug use. Increased maternal BMI is associated with increased birthweight (71). Maternal BMI is correlated with birthweight ($r=0.133$, $p<0.001$), with overweight mothers having babies who are on average 0.09 kg more, and obese mothers having babies who are on average 0.18 kg more than normal weight mothers' babies (71). The influence of physical activity on risk of LBW has also been evaluated. The two RCTs that tested the impact of exercise interventions on previously sedentary women during pregnancy and looked at the impact of these interventions on gestational length also looked at the impact of exercise on the risk of having an LBW infant (44,45). Haakstad examined the effects of going to aerobic dance classes on maternal and neonatal outcomes (44). Calculations from prior studies suggested that Haakstad et al. had the necessary number of participants to detect a 10% difference in birthweight with a power of 0.80 and alpha level of 0.05 (44). However, this study found no statistically significant difference in mean birthweight between the two trial groups (44). Nascimento et al., who looked at the impact of an exercise intervention

on overweight and obese pregnant women, also found no significant differences in the newborns' weight between the exercise and control groups (45).

Maternal SES has been linked with LBW (50,72,73). Low SES and prevalence of LBW were found in the 2010 systematic review of socioeconomic differences in negative birth outcomes conducted by Blumenshine (50). Considering the four pillars of SES used in this review, income, education, occupational status, and area-based measures had a majority of studies with significant associations between these measures and LBW, either in the whole study sample or in a subgroup (50). Common law marriage, compared to marriage, has been shown to have a negative impact on birthweight (72). Furthermore, a Brazilian study including 145,870 live born singleton infants found low maternal schooling to be associated with LBW in a multilevel model, with a p value <0.001 (73). This same study found that the occupational status of 'housewife' had an OR of 1.13 (95% CI 1.07-1.20) for LBW compared to 'other' using the overall model, including interaction terms (73).

Race and ethnicity have been identified as risk factors for LBW. Being Black is associated with adverse neonatal outcomes, including LBW (56). African-Americans in the United States have twice the risk of having an LBW infant compared to Whites (51). The United States National Vital Statistics showed that 13.5% babies born to blacks were LBW, whereas 7.1% born to whites and 7.0% born to Hispanics were LBW (74).

Seasonal variation in birthweight has been repeatedly demonstrated (58,75,76). Several environmental factors undergo seasonal variation, such as temperature, precipitation, and ultraviolet exposure (76). A Japanese study discovered a 12-month

rhythm for absolute birthweight, with peaks in May and October and troughs from June to September and also in December (58). A cohort study conducted over a decade and a half in Northern Ireland also found seasonal fluctuations in birthweight, with the late spring and summer births having the lowest mean birthweights (75). The authors of this study point out that these births were in their second trimester during the winter (75). This particular study found that adjusting for mean daily maximum temperature during the second trimester attenuated the seasonal variation (75). An Australian study examining 350,171 singleton pregnancies that excluded PTB also found yearly fluctuations of mean birthweight (76). The six-month difference for weight was 13 g for baby boys and 7 g for baby girls (76).

Alcohol consumption during pregnancy has been implicated in LBW (60). A recent review identified a study in which maternal consumption of alcohol during pregnancy was associated with decreased birthweight starting at two drinks per day (60). Use of illicit drugs such as opioids, marijuana, and stimulants during pregnancy can have damaging effects on infant birthweight (63). Cocaine, for example, has been shown to increase the odds of having a LBW infant, with an OR of 3.66 (95% CI 2.90-4.63) (63). The same systematic review and meta-analysis also found that using cocaine while pregnant decreased the offspring's weight by an average of 494 g (95% CI 421-562 g lower) (63).

Consequences/ Impact of Low Birthweight

Being born with LBW has negative consequences in the neonatal period and beyond. Infants weighting less than 2500 g at birth have a higher risk of mortality and

morbidity in the neonatal period compared to infants weighing at least 2500 g (77). Later in life, LBW is linked to increased risk of type II diabetes as well as coronary heart disease (CHD) compared to normal birthweight (78). Harder et al. performed a meta-analysis which found a U-shaped relationship between birthweight and risk of type II diabetes. Eriksson et al. found that the risk of CHD is associated with small body size at birth as a result of growth restriction rather than prematurity (79).

Vitamin D and Low Birthweight (Objective 1b)

One possible explanation for a protective association between vitamin D and LBW is vitamin D's influence on skeletal growth (80,81). The role of vitamin D may begin early on in pregnancy. Although fetal growth peaks in the third trimester, the growth trajectory of the fetus is set before this time in pregnancy, possibly in part by the vitamin D environment (82).

Observational studies examining maternal vitamin D in relation to birthweight have found higher vitamin D to be protective of LBW in infants (24,25). Data from a prospective cohort called the Collaborative Perinatal Project conducted across twelve medical centres in the U.S. with enrolment from 1959-1965 was used to evaluate the relationship between maternal vitamin D and infant birthweight, with Gernand as the principal investigator (24). White, Black, and Puerto Rican mothers with no prior history of diabetes or hypertension, who began prenatal care by 26 weeks gestation, had stored serum by 26 weeks gestation, and gave birth to a baby between 20-42 weeks were included (24). This nested study excluded mothers with stillbirth, PTB, or whose serum could not be assessed for vitamin D (24). The authors used liquid chromatography-

tandem mass spectrometry to evaluate circulating 25(OH)D after the serums had been stored for 40 years at -20°C. Pilot testing was conducted to verify the possibility of 25(OH)D degradation and this occurrence was deemed unlikely (24). Birthweight of the newborns was taken right after birth. This study found that mothers with vitamin D status ≥37.5 nmol/L had babies with higher birthweights than mothers with levels < 37.5 nmol/L (24). The relationship that they observed was nonlinear. Infant birthweight increased by increments of 3.6 g (95% CI 1.1-6.1) per 1 nmol/L increase in the mother's 25(OH)D up to maternal vitamin D status of 37.5 nmol/L (24). After 37.5 nmol/L birthweights levelled off in relation to maternal vitamin D. One major strength of this study was its exclusion of pregnancies with missing covariates, which only eliminated 167 pregnancies. Hence the authors were able to do a complete data analysis. Another strength was the extensive examination of covariates, especially considering the time period of the cohort.

Another study looked at the impact of maternal vitamin D status on newborns in a Chinese population (25). There were 70 participants in this study, all of whom were nulliparous pregnant women who gave birth to healthy singleton babies at full term. There were 58 cord blood samples provided. Vitamin D status was classified as severe vitamin D deficiency (<25 nmol/L), mild vitamin D deficiency (25-<50 nmol/L), insufficient (50-75 nmol/L), and sufficient (>75 nmol/L). More than 90% of the mothers and newborns included in this study had 25(OH)D levels below 50 nmol/L, and no participants or their offspring had vitamin D status above 75 nmol/L (25). Mothers with severe vitamin D deficiency gave birth to newborns with lower mean birthweight than

mothers with mild vitamin D deficiency (25). There were trends for an association between cord blood vitamin D and birthweight.

A multiethnic study analyzing 2739 participants found an association between maternal vitamin D serum levels in the first trimester and birthweight (28). The deficient group [less than or equal to (≤) 29.9 nmol/L] had lower birthweights than the sufficient group (≥50 nmol/L), with a difference of -114.4 g (95% CI -151.2, -77.6).

The current literature on cord blood vitamin D and LBW does not show a strong association between the two. Bowyer evaluated 901 umbilical cord blood samples, 604 of which were paired with maternal serums (4). Mothers who were vitamin D deficient gave birth to lower-weight babies than mothers with insufficient and sufficient levels, by 151 g after adjusting for potential confounding variables (4). Cord blood 25(OH)D and neonatal birthweight were not found to be related (4). Camargo also found no association between cord blood vitamin D status and birthweight (26). Hossain et al. found a negative relationship between cord blood vitamin D status (25-hydroxy vitamin D3) and birthweight ($r = -0.23$; $p = 0.048$) (5).

The major limitations of the study by Gernand (24) were that most of the participants enrolled during the second trimester, which prevented the authors from capturing the impact of vitamin D across the entire pregnancy, the fact that nearly half of the mothers reported smoking, and the inclusion of certain mothers more than once, as there were 2146 singleton births to 2096 mothers, presumably to mothers who had more than one pregnancy during the data collection time-frame. The lack of statistical significance despite the trend of higher cord blood vitamin D and birthweight in the study

on the Chinese population (25) is a limitation that the current study could fill with its higher numbers of cord blood samples. The exclusion of unhealthy and preterm babies prevented this study from capturing the full effects of vitamin D on birthweight, which will not be the case for the current study.

The Bowyer 2009 study classified ≤25 nmol/L as deficient and 26-50 nmol/L as insufficient (4). This classification may have provided too narrow a range of exposure levels to observe differences in the risk of LBW. In addition to this, the authors admitted that the assay techniques that they used may have overestimated the vitamin D status of the participants (4). The low number of only 75 participants in the Hossain study, of whom almost 90% had vitamin D status below 75 nmol/L, and 26% of whom covered their arms, hands, and head, presumably according to cultural practices, put into question the applicability of this study across populations without these practices (5).

In sum there are mixed results for the association between maternal as well as cord blood vitamin D status and birthweight. The current study aims to clarify this relationship by addressing challenges faced by previously conducted studies. The current study will improve upon the previous studies because the population is expected to have a much lower smoking prevalence than the 1959-1965 population in the Gernand study. This will improve upon any residual confounding that the large proportion of smoking caused in the previous study. The current study will also address previous problems of statistical significance with its greater power to detect a difference in risk of LBW based on vitamin D status because of the large number of cord blood samples included. Finally, the Canadian population being examined provides an opportunity to examine the

relationship between cord blood vitamin D and risk of LBW in a Northern population with cultural practices that differ from those in previous studies.

2.3.3 Small for Gestational Age

Prevalence and Risk Factors

In Canada in 2004 7.8/ 100 singleton live births in were SGA (<10^{th} percentile) (42). This measure has the same pitfalls of potential minor inaccuracies as PTB with regards to gestational dating. Potential risk factors for delivering an SGA infant include BMI, smoking, physical activity, maternal socioeconomic status (in general, marital status, education, occupation, and family income), ethnicity, emotional distress, season of measurement, alcohol, and illicit drug use. Both low and high maternal BMI increase the risk of having an SGA infant compared to normal maternal BMI (83). Low pre-pregnancy maternal BMI increases the risk for SGA among term babies (83). BMI equal to or above 30 kg/m^2 increases the prevalence of preterm SGA births (83). Both overweight and underweight mothers have augmented rates of moderate preterm and term SGA (83).

Maternal cigarette smoking during pregnancy accounts for 30-40% of the occurrence of SGA deliveries (42). Maternal smoking decreases various measures of newborn size, including weight, length, as well as chest and head circumference (84). Full-term infants born to mothers who smoked during pregnancy weigh an average of 300 g less than those born to mothers who did not smoke during pregnancy (84).

Disparities in the pervasiveness of SGA infant status based on maternal socioeconomic status (SES) emerged in a recent systematic review of SGA and

intrauterine growth restriction (IUGR) (50). Marital status has been shown to impact the prevalence of SGA in Quebec (49). Babies born to mothers who were married had a rate of 7.3% for SGA, those born to mothers cohabiting with their partners had a rate of 8.6%, and those born to mothers who were single had a rate of 11.8% (49). Education level, another component of SES, has also been shown to impact the risk of having an SGA infant. In simple logistic regression, the odds of SGA were 1.8 higher for babies born to mothers with 0-3 years of education compared to ≥12 years in a Brazilian population (73). Increased rates of SGA with decreasing levels of education have also been found to persist across Quebec (49). Among Canadian-born mothers in Quebec, being in the lowest area income tertile has been shown to have an odds ratio of 1.13 (95% CI 1.06, 1.20) for SGA compared to mothers in the highest area income tertile (49).

Maternal consumption of alcohol during pregnancy has been associated with SGA infant outcomes (60). This has been shown beginning at 3 drinks per day (60). Illicit drug use during pregnancy increases the risk of delivering an infant who is SGA (61,63). Opioid use has been shown to increase the odds of having an SGA infant, with an adjusted OR (aOR) of 1.9 (95% CI 1.7-2.1), as has marijuana use, with an aOR of 2.0 (95% CI 1.7-2.2) (61). Cocaine use during pregnancy has shown even stronger effects on the risk of this outcome, with an OR of 3.23 (95% CI 2.43-4.30) (63).

Consequences/ Impact of Small for Gestational Age Birth

Being born SGA increases the risk of perinatal mortality, as well as morbidity including metabolic disturbance, respiratory problems and thermoregulatory disturbances

(85). In the long-term, this also increases risk of morbidity for chronic problems such as obesity, hypertension and type II diabetes (85).

Vitamin D and Small for Gestational Age (Objective 1c)

Several observational studies have examined maternal vitamin D and SGA infants. A study conducted in England using <25 nmol/L during the 3rd trimester as its 25(OH)D cut-off and the <10th percentile derived from a large UK reference sample as its SGA measure did not find an effect of vitamin D status on risk of SGA infant (86). An additional study from a South Indian population that used 3rd trimester maternal serum with a <50 nmol/L cut-off had no observed effect of maternal vitamin D on triceps skin-fold, sub-scapular skin-fold, crown-heel length or placental weight of infants (87). The measures for SGA in this study were very extensive as they included size measurements as well as placental weight. A Dutch study involving 2739 participants, examining 1st trimester maternal serum with deficiency defined as ≤29.9 nmol/L and insufficiency as 30-49.9 nmol/L did observe an effect of maternal vitamin D on the risk of an infant below the 10th percentile of the most recent Dutch reference values (2008) for that gestational age according to sex (28). Deficient vitamin D status was associated with an aOR of 1.9 (95% CI 1.4-2.7) for having an SGA infant compared to the vitamin D sufficient group (28). An Australian study that excluded women with dark skin and took samples from expectant mothers during the 3rd trimester found an association between maternal vitamin D and knee-heel length at birth (29). Finally, an English study that looked at maternal vitamin D serum taken between 11 and 13 weeks gestation examined participants of African racial origin separately from Caucasians. This study found an effect of vitamin D on risk of being below the 5th percentile for gestational age in

Caucasians, but not in participants of African descent (30). This case-control study did an excellent job at controlling for potentially confounding variables and having complete outcome data.

In a multicentre prospective cohort studying gestational factors and offspring health, Burris et al. found cord plasma 25(OH)D levels <25 nmol/L versus ≥25 nmol/L were associated with lower birthweight-for-gestational-age in both unadjusted and adjusted models (OR 4.64 [95% CI 1.61, 13.36] in adjusted analyses) (31). Mothers with second trimester 25(OH)D levels below 25 nmol/L had increased odds of delivering an SGA infant compared to mothers with levels ≥25 nmol/L (OR 3.17 [95% CI: 1.16, 8.63]) after adjusting for season of blood draw, maternal age, pre-pregnancy BMI, and race (31). The higher OR with the cord blood compared to the maternal blood serums may be indicative of cord blood serving as a more direct measure of fetal exposure. This study was particularly well conducted because it involved 2128 participants, all participants had complete outcome data, and there was a thorough assessment of covariates. In addition to the covariates that are predominantly included in analyses of vitamin D and neonatal outcomes, which are race/ethnicity, maternal age, smoking status, prepregnancy BMI, socioeconomic measures, this study also looked at gestational weight gain, parity, infant sex, dietary intake from a validated food questionnaire, gestational diabetes, and pre-eclampsia.

In sum, both maternal and cord blood have been shown to be associated with higher risk of SGA. This relationship has not been shown in a consistent manner, or in all subpopulations. This could be due to some of the limitations of the previously conducted studies. The Baker study only examined mothers aged 14-18 years old. Furthermore, it

addressed missing data quite poorly (27). The use of placental weight in the Farrant study (87) is questionable as placental weight is not directly correlated with birthweight (88). The exclusion of women with dark skin from the Australian study limits the reach of its findings (29). The current study aims to address these concerns. What is more, the higher OR with cord blood versus maternal blood in the Burris study emphasizes the need for further studies to confirm the impact of cord blood vitamin D on risk of SGA (31) without relying on maternal vitamin D as a proxy for levels received by the growing fetus.

2.4 Maternal Vitamin D and Cord Blood Vitamin D (Objective 2)

There is considerable evidence to suggest that the fetus is largely dependent on its mother for vitamin D. Circulating 25(OH)D from the mother reaches the developing fetus via the placenta (9). The placenta contains 1 α-hydroxylase, an enzyme that converts vitamin D into its active form (3,89). Additionally, maternal metabolism of vitamin D is increased during pregnancy in part due to the fetal demands for vitamin D, and so pregnant women have increased requirements for vitamin D (4,9).

Maternal vitamin D status has shown a strong correlation with neonatal vitamin D status. A prospective population study conducted in Sydney Australia by Bowyer et al. recruited 971 women before 28 weeks gestation (4). The investigators in this study collected maternal blood at 30-32 weeks gestation and examined maternal vitamin D status in relation to venous cord blood vitamin D status when the babies were born. They collected 901 umbilical cord blood samples, 604 of which were paired with maternal samples. Maternal and infant vitamin D status had a correlation of 0.74, $p<0.0001$.

Neonates had lower rates of deficiency (11%) and insufficiency (29%) compared to mothers (15% and 33% respectively). The neonatal median was 60 nmol/L (range 17-245) compared to 52 nmol/L among mothers (4). The major strength of this study was its use of the Fitzpatrick skin phototypes, six phototypes based on how untanned skin would react to direct exposure to the sun for 30-45 minutes, to classify maternal skin phototype. This allowed the study to isolate skin colour as a biological factor for vitamin D status instead of entangling it with the more cultural measure of ethnicity. Although the assay used has been shown to consistently show higher vitamin D status than other methods by approximately 31% (90), which is a potential limitation of this study, this would not have a substantial impact on the correlation between maternal and neonatal vitamin D as long as the levels were consistently overestimated among both mothers and neonates. The omission of information on dietary intake of vitamin D and calcium, 50 nmol/L cut-off for insufficiency, and fact that 68% of the participants wore a veil would also have little impact on a simple examination of the relationship between maternal and neonatal vitamin D. Overall, for the purpose of looking at the correlation between maternal and neonatal vitamin D, this study was well-conducted and useful.

The study by Hossain et al. was a prospective cohort study conducted through and inner-city tertiary-care hospital in Karachi, Pakistan (5). This study involved 75 participants and their neonates, and found a correlation between maternal vitamin D status to be correlated with newborn levels ($r=0.70$ with female newborns, 0.68 with male newborns, $p<0.001$). The fact that 26% of the participants covered their hands, arms and head, as well as Karachi's relatively southern latitude would not have any great influence over the correlation between maternal and neonatal vitamin D. Sachan et al. examined the

relationship between maternal and neonatal vitamin D among participants in Northern India (32). The investigators in this study collected 207 maternal blood samples before labour and 117 cord blood samples after delivery. The correlation coefficient between maternal and cord blood vitamin D was 0.70, $p<0.001$.

Dror et al. conducted a cross-sectional observational study in Oakland in the United States, involving 210 mother-infant pairs (35). Maternal blood was drawn upon admission to the labour and delivery unit. This study found a correlation of 0.79, $p<0.0001$ between maternal and neonatal vitamin D status. In addition to this, the authors reported that fetal levels were lower than their mothers' levels, at an average 61% ± 18% of their mothers' levels. Including this finding was unique to this paper. It is helpful for contributing to notions of whether or not fetal cut-offs ought to be based on adult cut-offs. If they should be the same as adult cut-offs then knowing the full relationship between maternal and fetal vitamin D (beyond just the correlation) is important in deciding what recommendations to provide pregnant women with so that they can achieve high enough levels for their babies to have optimal vitamin D status and health. The inclusion of a multiethnic population in this study enlarged the reach of its generalizability.

There are certain limitations worth noting from these studies. In the Bowyer study (4), the assay used and the sample population that included 68% of participants that wore a veil could influence the validity and generalizability of the study. The Hossain study (5) also faces issues of genearlizability due to the skin-covering practices of so many of its participants. This is particularly relevant with regards to how the results might eventually be used. Public health campaigns need to take into account the contexts in which their

populations live, and the aforementioned studies have populations with quite different cultural and environmental contexts compared to Canada.

One potential weakness of the study by Sachan et al. is that the women who agreed to participate in the study had newborns with significantly higher birthweight compared to women who declined (32). This may mean that the study population was healthier than the general population, which could undermine the external validity of the results if the included population were systematically different from, or simply not representative of the general population.

All of these studies found positive correlations between 0.68 and 0.79 between maternal and neonatal vitamin D. The limitations of these studies did not strongly hinder the results of the findings on this particular question. However, this secondary objective of the current study, looking at the relationship between maternal and neonatal vitamin D status, remains important to in order to examine whether or not the results are consistent with the literature, and so that insights can be provided for studies that only look at maternal or neonatal vitamin D. The current study will also enrich the existing literature by evaluating the linearity of the relationship between maternal and fetal vitamin D status before potentially calculating a correlation coefficient. Moreover, only the Dror et al. study elaborated on the exact relationship between maternal and neonatal vitamin D (35). This is much more useful and informative to report, and a gap that is otherwise missing in the literature. The current study will help to contribute to the understanding of this relationship.

2.5 Contribution of Current Study given the Existing Literature

Focussing on cord blood in relation to PTB, LBW and SGA is an improvement over maternal serums because it provides a better measure of the vitamin D available to the fetus. Assessing 25(OH)D in cord serums will also allow for the identification of whether or not there is a need for additional supplementation in neonates, especially those with PTB, LBW and/or SGA classifications. By also examining the relationship between maternal and cord serum vitamin D status this study was able to shed light on previously conducted studies that only looked at maternal vitamin D and neonatal outcomes.

The current study's large sample size from a general population with a 25(OH)D concentration <75 nmol/L prevalence of 63.7% should enable the detection of a clinically meaningful effect of low 25(OH)D on the neonatal outcomes of interest. This study has the ability to control for several important covariates, including BMI, smoking status, maternal age, physical activity before and during pregnancy, SES measures, ethnicity, emotional distress, season of measurement, alcohol intake, and drug use, which are not all consistently controlled for in the analysis of previous studies. The use of 25(OH)D to evaluate vitamin D and the particular assay techniques used to assess these levels for the purpose of this study will contribute to the reliability and validity of the results. This study will also be more generalizable to the Canadian and similar populations than several other previously conducted studies.

CHAPTER THREE: METHODS

3.1 Overview

The data for this nested case-control study included cord blood samples, maternal serum samples, questionnaires as well as maternal and neonatal medical records that were previously collected in Quebec City, Quebec. For the primary objective of this project vitamin D status in cord blood was the exposure, and PTB, LBW infant, and small-for-gestational-age infant were the outcomes. Using 25(OH)D levels, vitamin D was classified as 25(OH)D ≥75 nmol/L, 50 -<75 nmol/L, 37.5 -<50 nmol/L and <37.5 nmol/L based on the most up-to-date guidelines from Health Canada (34) as well as the Canadian Pediatric Society, Endocrine Society, and the current literature (35,91),(92).

For the secondary objective, maternal serum vitamin D status was the independent variable, and cord blood vitamin D status was the dependent variable. From the 6694 participants who completed the questionnaires for the original cohort, mother-baby pairs were classified as cases based on the three outcomes of interest for this study (PTB, LBW, and small-for-gestational-age) as well as pre-eclampsia, gestational diabetes, and spontaneous abortion for a total of 1378 cases. Controls were frequency-matched against all of the cases (including maternal outcomes examined elsewhere) based on gestational week of recruitment, month/ year of blood collection, and the absence of any of the six original case definitions. Cases with adverse neonatal outcomes and available cord blood as well as controls with available cord blood were selected for the purpose of this study.

3.2 Study Design, Inclusion Criteria

The current study was a nested-case control study. The data used was originally collected for a Canadian Institutes for Health Research (CIHR)-funded cohort study of pregnant women recruited before 20 weeks gestation in Quebec City for the purpose of creating a serum and fetal DNA bank. The focus of the present study was a subpopulation from the original study with fetal cord blood available.

Recruitment for this cohort took place between 2005 and 2010. A research nurse enlisted participants during their first routine prenatal visit to the hospital (between weeks 14 and 17 from 2005-2008, or weeks 8 and 12 from 2008-2010). Participants provided informed consent if they were willing to participate in the study. Of relevance for the current project, blood samples were collected at this initial visit, as well as at delivery, simultaneously with clinical blood collection. After delivery, a blood sample was drawn from the umbilical cord. All blood samples were aliquoted and stored at -80°C.

A flowchart of the study cohort in presented in Appendix 1. Of the women who were approached to participate in this study 85% agreed, for a total of 7929 women. Fifty-five participants withdrew. At recruitment 7855 blood samples were collected and 6694 questionnaires were completed. At delivery 5200 cord blood samples were collected.

A 24-page questionnaire (Appendix 2) that took approximately 15-20 minutes to complete was given out by a research nurse at a routine hospital visit during the second trimester to participants who had previously provided informed consent. The questionnaire was written in French and filled out in writing by the participant, with the

opportunity to ask the research nurse to clarify the questions being asked. Questions included in the study asked for information about the participants' demographics, physical measures, socioeconomic measures, living arrangements, lifestyle before and during pregnancy, gynecological and obstetrical history, medical history, family and child's father's family, and emotional distress.

Chart reviews (Appendix 3) that were completed after the pregnancy for each participant included information based on the patient and baby's hospital records, including maternal medical history, gynecological and obstetric history, history of current pregnancy, disease or disorders during this pregnancy, medication taken during this pregnancy, delivery, as well as information about the baby's birth, anthropometric data and clinical data. The clinical data provided information on stillbirth, admission to the intensive care unit, septicaemia, pneumonia, necrotic enteritis, haemorrhage, jaundice, other complications and breastfeeding in hospital. These chart reviews were used to identify the conditions that determined case status. Only cases that had cord blood were included for analysis in this study.

3.3 Case and Control Selection

3.3.1 Overview

Only live births were included in the study for both cases and controls. Pregnancy loss, including spontaneous abortion and stillbirth, are being examined elsewhere. Twins and triplet births were excluded because of their higher risk of PTB, LBW and SGA (93). This study separately compared each of the three case groups and a combined case group to the same control group.

3.3.2 Case Definitions

Cases were defined by PTB, LBW infant, and/or SGA infant and are defined below.

1) Preterm birth is a delivery occurring at <37 weeks gestation, with gestational age based on ultrasound information, if available, or else the last menstrual period.
2) A low birthweight infant is one weighing <2500 grams at birth.
3) A small for gestational infant is one born at the bottom 10^{th} percentile based on Canadian standards for sex specific birthweights by gestational week (94).

A total of 222 cases with PTB, 106 with LBW, and 301 with SGA classifications were included in this study.

3.3.3 Control Definition

Controls for the original cohort were frequency matched to all of the cases based on gestational week of enrollment (<10, 10-13, 14-15, 16-17), season (April-September, October-March) and the year that the mother was initially recruited for the study. For this particular study only controls with cord blood samples will be included. There were 1027 controls included.

All mothers with pre-eclampsia and/or gestational diabetes were included for 25(OH)D analysis in the larger study. Keeping all of these mother-baby pairs would lead to an over-representation of mothers with pre-eclampsia and/or gestational diabetes for the purpose of this study. Since some cases had these conditions, the controls were represented at the rate consistent with the overall cohort. Therefore only 1.2% of mothers

with pre-eclampsia and 5.2% with gestational diabetes were included among the controls in the study. The mother-baby control pairs where the mother had at least one of these conditions were chosen using a random selection process in SAS 9.2, with a different seed for pre-eclampsia and for gestational diabetes.

3.4 Exposure Assessment

Cord blood was collected for approximately 55% of the cohort participants. Infants with consenting mothers had a blood sample taken from the umbilical cord after delivery. Approximately 90 mL of umbilical cord blood was taken after the umbilical cord was collected. The sample was centrifuged to separate the serum from the cord blood, refrigerated for a number of hours, aliquoted in ~1mL tubes and frozen at -80°C for future analysis. When the maternal blood was drawn during the first trimester it was also separated immediately, refrigerated for a number of hours, and frozen in ~1mL tubes at -80°C. From the obtained maternal and cord serums, 25(OH)D levels were determined in Dr. Hope Weiler's lab at McGill University. The laboratory received a certificate of Proficiency for 2009-2010 from the DEQAS indicating that at least 80% of the reported results were no more than 30% outside of the all-laboratory mean (95). The laboratory used automated chemiluminesence assays to measure serum 25(OH)D from both the umbilical cord and the maternal samples.

3.5 Covariate Information

Based on the current literature and biological plausibility the following potential confounders, which could influence our exposure-disease relationship, were considered:

Body Mass Index (BMI): The questionnaire contained information on weight before pregnancy (written in pounds or in kilograms) and height [in feet and inches or in centimetres (cm)].Weight before pregnancy written in pounds was converted to kilograms. Height written in feet and inches was converted to cm. BMI was calculated based on the formula: BMI = (weight in kilograms)/[(height in metres)^2]. BMI was grouped according to the standard underweight (<18.5), normal (18.5-24.9), overweight (25-29.9), and obese (≥30) categories (96). BMI was categorized according to these groups instead of continuously because the influence of BMI on the risk of the neonatal outcomes that were examined is nonlinear (35,38). Since less than 10% of the total sample population was underweight this group was collapsed into the referent (normal) category.

Smoking: The questionnaire contained the classifications of non-smoker, ex-smoker, and current smoker. Ex-smokers were asked when they stopped smoking (during the 2nd trimester, during the 1st trimester, upon finding out about being pregnant, less than 6 months before pregnancy, 6 to 12 months before pregnancy, over a year before pregnancy). Smokers were asked how many cigarettes they smoke on average per day (less than 1 per day, 1 to 10, 11 to 25, 26 or more). Participants' smoking status was based on whether they smoked during pregnancy. Non-smokers and ex-smokers who had stopped smoking before pregnancy were considered non-smokers during pregnancy and combined to form the referent group. Smokers and ex-smokers who had stopped smoking upon learning of that they were pregnant or during the first or second trimesters were considered to be smokers at some point during pregnancy and grouped together.

Second-hand smoking exposure during pregnancy was classified based on the general question of exposure during pregnancy as very few respondents answered questions about exposure in the home, at work, or during leisure time. People exposed to second-hand smoke during pregnancy were compared to those who were not.

Maternal Age: Maternal age at delivery was based on the mother's date of birth and the baby's date of delivery. The categories for maternal age were selected based on knowledge that pregnancy in adolescence is associated with a higher risk of adverse neonatal outcomes, as is being over the age of 34, and additionally over age 39 (97-100) but also based on the numbers of participants in these categories in the dataset. Maternal age between from 25 to 34 years was used as the referent category, with maternal age below 25 years and maternal age equal to or above 35 years as the other two categories.

Physical Activity: The questionnaire contained information on the frequency of physical activity for 20-30 minutes during free time in the 3 months before pregnancy. Details on the type or intensity of physical activity were not provided. Participants who engaged in physical activity once or more a week were grouped together as the referent category, those who exercised 1-3 times per month were grouped together, and those who did not engage in physical activity once in the three months prior to pregnancy were categorized as completely sedentary (38). The frequency of physical activity for 20-30 minutes during free time in the last 3 months during pregnancy was also included in the questionnaire. Frequency of physical activity during pregnancy was grouped based on the same categories as pre-pregnancy physical activity (101).

Socioeconomic Status (SES): The SES factors that were included as potential covariates were marital status, education, and family income. A distinction between married and common-law partner within the subcategory of marital status was maintained based on previous research in Quebec (49), as described in the 'General Prevalence and Risk Factors for, and Consequences of Preterm Delivery, Low Birthweight and Small for Gestational Age' section of the literature review, and because these two categories had different associations with the outcomes of interest in the study cohort. Education was based on the respondent's highest educational qualification was categorized as: none (did not finish high school) amalgamated with completed high school (including vocational studies), completed college (Collège d'enseignement général et professionnel, CEGEP), and completed university. Occupation was not examined as a potential covariate. Without information on indoor/outdoor work, occupational information would not add much to the analysis as it would mainly be a proxy for income and possibly stress, for which better sources of information were available. Even information on shift work would not be particularly informative with regards to PTB, LBW and SGA based on a recent systematic review and meta-analysis (102). Family income was based on the categories included in the questionnaire (less than $15,499, $15,500 to $24,999, $25,000 to $39,999, $40,000 to $59,999, $60,000 or more, I don't know, I refuse to answer this question). The two lowest income categories were combined. Answers "I don't know" and "I refuse to answer this question" were treated as missing data.

Ethnicity: Frequency tables were created for the ethnicity categories in order to assess the prevalence of different ethnicities in the study population based on the mother's parents (Caucasian [white], First Nations, African-Canadian, Asian, don't

know, other). The study population was categorized as Caucasian (white) and 'other' based on the low numbers found outside of the Caucasian population, and also based on the higher risk of vitamin D deficiency in non-Caucasians (38).

Emotional Distress: Emotional distress level was assessed with 23 questions asking about the week prior to completing the questionnaire. Questions included information on anxiety and depression properties such as "Did you feel worried, troubled or anxious?" and "Did you feel you had a great weight on your shoulders?" with responses of how often ranging from never, rated as 1, to very often, rated as 4. The Hospital Anxiety and Depression Scale (HADS) uses similar questions and groups results as normal, borderline abnormal (borderline case) and abnormal (case) based on scores of 0-7, 8-10, and 11-21, separately for depression and anxiety. The HADS is not recommended as a research tool because the trade-off between its sensitivity and specificity does not allow for a clear-cut assessment of anxiety and depression (103). With the subscale cut-off of 8, the anxiety subscale has 71% sensitivity and 45% specificity, while the depression subscale has a sensitivity of 31% and specificity of 66% (103). Moving the cut-point in this scale does not improve this measurement tool (104). For the purpose of this study, an emotional distress score for each participant was calculated and used as a continuous measure. There were two questions with reversed scoring ("Did you feel relaxed?" and "Did you feel full of energy, in good form?"). The continuous score was therefore based on the number of negative emotion questions (n=21) multiplied by their occurrence on the 1-4 scale, minus the number of positive emotion questions (n=2) multiplied by how often they had happened based on the 1-4

scale. Hence the best possible score was 13 [(21*1) – (2*4) = 13] and the worst possible score was 82 [(21*4) – (2*1) = 82].

Season of Measurement: Season of maternal blood sampling and season of delivery for cord blood were categorized based on a one-month lag (35) of the approximate solstices (June 21st and December 21st) and approximate equinoxes (March 21st and September 21st). Hence four categories were created: late summer/ early fall (July 21st- October 20th), late fall/ early winter (October 21st- January 20th), late winter/ early spring (January 21st-April 20th), and late spring/ early summer (April 21st- July 20th).

Alcohol: The questionnaire included information on how much alcohol the respondent consumed per day or per week on average before and during pregnancy. Since the categorization of alcohol consumption during pregnancy is inconsistent (60) and the Society of Obstetricians and Gynaecologists of Canada recommends zero consumption of alcohol during pregnancy (105), the prevalence of alcohol consumption during pregnancy was examined initially. Ultimately alcohol was not included for further analysis as the prevalence of drinking alcohol in the week before completing the questionnaire was too low. Nearly 75% of participants did not answer this question. Of those who did, only 6.3% had one drink per week and less than 1% consumed alcohol to a greater extent than that.

Drug Use: Drug use has been broken down in the questionnaire based on the use of illegal drugs, the type of drug(s) used, the time-period of use, and the frequency of use

if the respondent was using at the time. Drug use was not explored beyond initial frequencies as only 15 women reported any drug use during pregnancy.

Gestational Age (for LBW): Based on the recommendation from Delbaere et al. (106), gestational age was not adjusted for when looking at LBW. This way the total effect of vitamin D status on LBW can be captured.

3.6 Statistical Analysis: Initial Steps

All statistical analyses were performed in SAS 9.2 (Cary, NC). The significance for all statistical tests was set at a p-value of 0.10. Graphs were produced in Microsoft Excel using data from SAS outputs or directly in SAS. Vitamin D status was classified as ≥75, 50-<75, 37.5-≤50 and < 37.5 nmol/L based on the Health Canada, the Canadian Pediatric Society (41), Endocrine Society, and the current literature (35,91,92). Since no study participants had 25(OH)D concentrations above 225 nmol/L, this level was not a considered in this study.

Preliminary analyses included searching for any obvious outliers with consideration given to whether or not these could be errors based on their plausibility. Errors were rectified in consultation with collaborators in Quebec. The number of cases, number of controls, missing data, and way in which the variables were coded was examined. Frequency tables were used to examine the categorical variables, means and standard deviations for normally distributed continuous variables, and medians and interquartile ranges for non-normally distributed continuous variables. Age and BMI were recoded into categories based on the literature in terms of biological plausibility, clinical relevance, and convention, as mentioned previously. When there were low numbers in a

particular category of a potential confounder and adjacent categories confounded the relationship between the exposure and outcome in a similar manner, these categories were collapsed.

Participant characteristics (exposure and covariates) were described by outcome status. Among controls, covariates were described by exposure status [25(OH)D category]. The cases and controls were compared with means for continuous variables using Student's t-tests and with proportions for categorical variables using chi-square tests.

3.7 Modeling Strategy Addressing the Primary Objective: Is Vitamin D Status in Cord Blood at Birth Associated with Preterm Delivery, Low Birthweight, and Small for Gestational Age?

The magnitude of the crude associations between vitamin D status (each level compared to the referent of ≥75 nmol/L) and PTB, LBW, and SGA outcomes was determined separately and for all three adverse neonatal outcomes combined after ensuring the data were properly formatted to calculate the odds ratios (ORs). ORs and 95% confidence intervals (CIs) were determined. Crude ORs for neonatal case status (PTB, LBW and/or SGA) versus control status were calculated using the <37.5, 37.5-50, 50-<75 nmol/L groupings, each compared to the referent of ≥75 nmol/L. Crude ORs above and below each cut-point (37.5, 50, 75 nmol/L) were also calculated for each adverse neonatal outcome of interest.

Potential confounders were assessed for their effect on the association of 25(OH)D and each neonatal outcome. Model building took a forward approach, assessing the

percentage change that each potential confounder made to the relationship between vitamin D status and the outcome. In other words, the adjusted odds ratio (aOR) for the association between 25(OH)D and the outcome (determined from the model including the potential confounder) were compared to the crude OR (determined from the model without the potential confounder). Crude ORs used for comparison with aORs were calculated among the subgroup that contained only complete data for the potential confounder of interest. Variables that contributed at least a 5% difference between the aOR and crude OR were considered to be confounders and were included in the model. The exposure-neonatal outcome associations were examined, adjusting for potential confounders simultaneously, using logistic regression to estimate adjusted ORs and 95% CIs.

The presence of effect modification between the main exposure [i.e. 25(OH)D level] and main outcomes was assessed for each potential effect modifier. Potential effect modifiers of interest included maternal BMI, smoking, and age. Each logistic regression model assessing effect modification included the main exposure, confounders, and the particular interaction term being evaluated (e.g. 25(OH)D*effect modifier). The significance of each interaction term was determined using the Wald chi-square test with degrees of freedom equal to the number of levels of the main exposure minus one multiplied by the number of levels of the putative effect modifier minus one. Interaction terms with a p-value of 0.1 or smaller were explored further by examining the association between 25(OH)D and the outcome within strata of the effect modifier.

Since ≥ 10% of the data on several covariates was missing, multiple imputation (MI) was used to estimate the missing values (107). Markov Chain Monte Carlo (MCMC)

procedure was used for the imputations. Using the MCMC method, the missing values were estimated one hundred times based on available covariates, and then the mean of the estimated values was used for the final calculations. This method allowed for unbiased estimates of the means and variances. Categorical variables were recoded as dummy variables for imputation.

3.8 Statistical Analysis Addressing the Secondary Objective: Examining the Relationship between Maternal Vitamin D Status During the First Trimester of Pregnancy and Vitamin D status in Fetal Cord Blood at Birth

A scatter-plot was created to illustrate the relationship between maternal 25(OH)D levels during the first trimester and neonatal 25(OH)D levels at birth among controls. The scatter-plot was visually assessed for linearity and general trends. Linearity was also assessed with the graphical examination of residuals from a linear regression of fetal 25(OH)D on maternal 25(OH)D among controls. The correlation coefficient, r, of the association between maternal and cord vitamin D status and parametre estimates were computed. Month of maternal blood draw, maternal pre-pregnancy BMI, infant sex, maternal smoking during pregnancy, time between maternal and neonatal blood draw, and case-control status were tested as potential confounders and effect modifiers using forward and backward step-wise regression. The impact of each term was determined using the p-value (≤ 0.10) from the Wald Chi-square test. The average difference and average percentage variation between maternal serum and cord blood vitamin D status were calculated. Results stratified by sex of the baby, maternal pre-pregnancy BMI

category, maternal smoking during pregnancy, season of delivery were calculated and presented in tables. To test whether or not the relationship between maternal and fetal 25(OH)D was significantly different among the strata of these variables the interaction between each of these covariates and neonatal 25(OH)D was evaluated using a p-value of <0.10 as the cutoff for statistical significance. Results were also stratified by case/ control status. These results were presented in tables. To see if the relationship between maternal and cord 25(OH)D differed significantly by PTB, LBW and SGA status the interaction between each of these adverse neonatal outcomes and neonatal 25(OH)D was assessed using a p-value of <0.10 as the cutoff for statistical significance.

3.9 Smallest Significant Odds Ratio Given Sample Size

Because the sample size for this study was fixed, the smallest detectable significant odds ratio was calculated a priori for our sample size for each of the three neonatal outcomes separately using the power calculator for unmatched case-control studies in OpenEpi (108). A population prevalence of vitamin D status below 75 nmol/L was assumed to be 63.7% based on women ages 20-39 in the 2007-2009 Canadian Health Measures Survey as reported (14). It was assumed that between 45 and 55% of cases would have a cord blood sample. The best case scenario under this assumption would have 268 cases with PTB, 174 with LBW and 258 with SGA. The conservative scenario would have 220 cases with PTB, 143 with LBW and 211 with SGA. Both scenarios assumed that there would be 750 controls with cord blood available. The tests used to calculate the smallest significant ORs given the fixed sample size had approximately 80% power (alpha = 0.05) and was two-tailed.

The ORs below signify the smallest statistically significant odds ratio that could be detected under the given assumptions. An odds ratio of 1.5 for the <75 nmol/L group compared to the ≥75 nmol/L group for PTB would mean that pregnant women with vitamin D <75 nmol/L have 1.5 the odds of delivering a PTB compared to the referent group, for example. Considering the impact of our three outcomes on the lives of newborns and also taking into account the current literature on these topics, ORs of 1.5 or more should were considered both clinically significant and reasonable to expect (21,24,25,28-31). Moreover, this cut-off for comparison is also in-line with the goal of looking at vitamin D status beyond the focus of the impact on bone-related functions.

Table 3.1: Smallest Significant Odds Ratio Given Sample Size

		Smallest Detectable Significant Odds Ratio Best Scenario: 55% of Cases with Cord Blood			**Smallest Detectable Significant Odds Ratio Conservative Scenario: 45% of Cases with Cord Blood**		
Exposure	**Prevalence in controls (n = 750)**	**PTB (n=268)**	**LBW (n=174)**	**SGA (n=258)**	**PTB (n=220)**	**LBW (n=143)**	**SGA (n=211)**
≥75 nmol/L Reference (ref.)	36.3%	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
<75 nmol/L	63.7%	1.5	1.7	1.6	1.6	1.8	1.6

3.10 Ethics

Research Ethics Board approval was given to investigators in Halifax and Quebec City for conducting 25(OH)D analyses on the stored blood samples from the cohort participants.

CHAPTER FOUR: RESULTS

4.1 Chapter Overview

This chapter is divided into five subsections. These subsections correspond to the three neonatal outcomes of interest, each addressed individually, all three neonatal outcomes combined, and finally the association between maternal 25(OH)D drawn in the first trimester and umbilical cord 25(OH)D drawn at birth. For each of the first four subsections, the study population is described and results are presented for the complete case analysis, multiple imputation analysis, and interactions. The final subsection in this chapter includes results pertaining to the relationship between maternal vitamin D and neonatal vitamin D.

4.2 Potential Confounders among Controls by Categorized 25(OH)D

Potential confounders among controls by categorized 25(OH)D concentrations are presented in Table 4.1. Differences in maternal age based on neonatal 25(OH)D concentrations were found to be statistically significant ($p<0.01$). Mean maternal age was highest among controls with 25(OH)D concentrations ≥75 nmol/L (30.1 ± 4.1 nmol/L) and lowest among controls with 25(OH)D concentrations 37.5-<50 nmol/L (28.8 ± 3.9). However, differences in maternal age based on neonatal 25(OH)D were no longer statistically significant when age was categorized ($p = 0.43$). Vitamin D status differed by categorized and continuous BMI ($p=0.05$ and $p=0.02$). A higher percentage of neonates whose mothers had a normal or healthy BMI pre-pregnancy had vitamin D status ≥75 nmol/L compared to neonates whose mothers had a BMI 25-<30 or ≥30 pre-pregnancy (35.8%, 30.5% and 22.4%). A lower percentage of neonates whose mothers had a normal

or healthy BMI pre-pregnancy had vitamin D status 37.5-<50 nmol/L versus those who mothers had a BMI that was 25-<30 or ≥30 pre-pregnancy (13.8%, 15.5% and 16.8%). Mean maternal pre-pregnancy BMI decreased across the four 25(OH)D concentration categories (23.5 ± 4.7, 24.7 ±5.4, 24.6 ± 5.6 and 24.9 ± 5.7 kg/m^2 for 25(OH)D ≥75, 50-<75, 37.5-<50 and <37.5 nmol/L). Exposure to second-hand smoke was associated with lower vitamin D status (p=0.09). A lower percentage of offspring of mothers who reported exposure to second-hand smoke during pregnancy had 25(OH)D concentrations ≥75 and 50-<75 nmol/L compared to offspring of mothers who reported no exposure to second-hand smoke during pregnancy [30.9% versus (vs.) 34.3% and 40.0% vs. 43.8%].

Pre-pregnancy physical activity was associated with higher levels of vitamin D (p<0.01). There were 33.6% of neonates whose mothers had exercised at least twice a week before pregnancy who had 25(OH)D concentrations ≥75nmol/L, whereas only 27.3% of neonates whose mothers did not exercise once in the time before pregnancy had 25(OH)D concentrations ≥75 nmol/L. Consistent with this pattern, 7.5% of neonates whose mothers had exercised at least twice a week pre-pregnancy had 25(OH)D concentrations <37.5 nmol/L, whereas 16.7% of neonates whose mothers had not exercised at all pre-pregnancy had 25(OH)D concentration below this cut-point. Similar results were found for maternal physical activity during pregnancy and neonatal vitamin D status (p<0.01). A higher percentage of babies whose mothers had exercised at least twice a week during pregnancy had vitamin D status ≥75 or 50-<75 nmol/L than babies whose mothers had not exercised at all during pregnancy (33.4% vs. 27.6% and 44.8% vs. 36.6%). A lower percentage of babies whose mothers had exercised at least twice a week during pregnancy had vitamin D status 37.5-<50 and <37.5 nmol/L than babies

whose mothers had not exercised once during pregnancy (14.2% vs. 17.1% and 7.6% vs. 18.7%).

Maternal marital status was related to vitamin D status in the umbilical cord samples ($p=0.07$). Mothers who were married or common law with their partner both had a higher percentage of offspring with 25(OH)D levels in the highest vitamin D category (27.4% and 34.1% vs. 13.9%). These women also had lower percentages of their offspring with 25(OH)D concentrations <37.5 nmol/L compared to single or separated/ divorced mothers' offspring (11.5% and 10.0% vs. 13.9%). Higher levels of education were associated with higher levels of vitamin D ($p=0.06$). The percentage of babies with 25(OH)D concentrations ≥75 nmol/L was highest among infants of mothers with a university degree and lowest among infants whose mothers' educational level was high school or no high school (36.7% and 26.4%). The percentage of babies with 25(OH)D concentrations <37.5 nmol/L was smallest among infants of mothers with a university degree (8.7%) and largest among infants of mothers who had completed CEGEP (10.7%). Higher family income was associated with higher levels of vitamin D ($p<0.01$). The percentage of infants with 25(OH)D concentrations ≥75 nmol/L was highest among babies born to a family with an income ≥60,000 (40.2%) and this percentage decreased with each lower family income category (27.8%, 27.3% and 20.7% for family incomes of $40,000-<$60,000, $25,000-<$40,000 and <$25,000, respectively). Babies born to families with incomes <25,000 had a much higher percentage of 25(OH)D concentrations <37.5 nmol/L compared to any other category of income (20.7% vs. 6.3%, 9.8% and 8.1% when family income was $25,000-<$40,000, $40,000-<$60,000 and ≥$60,000, respectively). Babies born to Caucasian mothers had a higher percentage of 25(OH)D

concentrations in the highest category compared to other ethnicities (34.3% vs. 12.8%) and had a lower percentage of 25(OH)D concentrations in the lowest category compared to other ethnicities (8.9% vs. 25.6%; $p<0.01$). Differences in 25(OH)D concentrations were observed based on season of birth ($p<0.01$). The proportion of babies with 25(OH)D concentrations ≥75 nmol/L was highest among those born in the late summer/ early fall had, followed by babies born in the late spring/ early summer, late fall/ early winter, and finally late winter/ early spring (57.5%, 35.3%, 21.1% and 13.8%, respectively). The percentage of babies with 25(OH)D concentrations <37.5 nmol/L was lowest among babies born in late summer/ early fall, then late spring/ early summer, late fall/ early winter, and highest among babies born in late winter/ early spring (1.6%, 5.9%, 12.7% and 21.5%).

Table 4.1: Potential Confounders among Controls by Categorized 25(OH)D*

Variable	**Level**	**≥75 nmol/L n=334**	**50- <75 nmol/L n=437**	**37.5- <50 nmol/L n=153**	**<37.5 nmol/L n=103**	**P-value**
Age	(Continuous)	30.1(4.1)	29.5 (4.3)	28.8 (3.9)	29.1 (4.2)	<0.01
Categorized age (years)	<25 25-<35 ≥35	30 (25.0%) 259(33.2%) 45 (35.7%)	57 (47.5%) 324 (41.5%) 56 (44.4%)	18 (15.0%) 121 (15.5%) 14 (11.1%)	15 (12.5%) 77 (9.9%) 11 (8.7%)	0.43
Categorized BMI (kg/m^2)	<25 25-<30 ≥30 Missing	229 (35.8%) 57 (30.5%) 28 (22.4%) 20 (26.3%)	267 (41.8%) 76 (40.6%) 63 (50.4%) 31 (40.8%)	88 (13.8%) 29 (15.5%) 21 (16.8%) 15 (19.7%)	55 (8.6%) 25 (13.4%) 13 (10.4%) 10 (13.2%)	0.05 0.37**
BMI	(Continuous)	23.5 (4.7)	24.7 (5.4)	24.6 (5.6)	24.9 (5.7)	0.02
Smoking during pregnancy	No Yes Missing	245 (32.5%) 63 (35.4%) 26 (27.4%)	328 (43.5%) 70 (39.3%) 39 (41.1%)	108 (14.3%) 29 (16.3%) 16 (16.8%)	73 (9.7%) 16 (9.0%) 14 (14.7%)	0.70 0.32**
Second-hand smoke exposure	No Yes Missing	212 (34.3%) 105 (30.9%) 17 (25.0%)	271 (43.8%) 136 (40.0%) 30 (44.1%)	79 (12.8%) 62 (18.2%) 12 (17.7%)	57 (9.2%) 37 (10.9%) 9 (13.2%)	0.09 0.48**

Variable	Level	≥75 nmol/L n=334	50- <75 nmol/L n=437	37.5- <50 nmol/L n=153	<37.5 nmol/L n=103	P-value
Physical activity pre-pregnancy	2 or more times/ week	237 (33.6%)	313 (44.3%)	103 (14.6%)	53 (7.5%)	<0.01
	1-3 times/ month	65 (34.2%)	66 (34.7%)	29 (15.3%)	30 (15.8%)	
	Not once	18 (27.3%)	28 (42.4%)	9 (13.6%)	11 (16.7%)	
	Missing	14 (21.5%)	30 (46.2%)	12 (18.5%)	9 (13.9%)	0.22**
Physical activity during pregnancy	2 or more times/ week	217 (33.4%)	291 (44.8%)	92 (14.2%)	49 (7.6%)	<0.01
	1-3 times/ month	69 (36.5%)	70 (37.0%)	28 (14.8%)	22 (11.6%)	
	Not once	34 (27.6%)	45 (36.6%)	21 (17.1%)	23 (18.7%)	
	Missing	14 (21.2%)	31 (47.0%)	12 (18.2%)	9 (13.6%)	0.21**
Marital status	Married	43 (27.4%)	77 (49.0%)	19 (12.1%)	18 (11.5%)	0.07
	Common law	157 (34.1%)	186 (40.4%)	71 (15.4%)	46 (10.0%)	
	Single or separated/ divorced	5 (13.9%)	17 (47.2%)	9 (25.0%)	5 (13.9%)	
	Missing	129 (34.5%)	157 (42.0%)	54 (14.4%)	34 (9.1%)	0.72**
Education attainment	University	139 (36.7%)	166 (43.8%)	41 (10.8%)	33 (8.7%)	0.06
	CEGEP	115 (34.1%)	133 (39.5%)	53 (15.7%)	36 (10.7%)	
	High school or did not complete high school	55 (26.4%)	94 (45.2%)	38 (18.3%)	21 (10.1%)	
	Missing	25 (24.3%)	44 (42.7%)	21 (20.4%)	13 (12.6%)	0.14**
Family income	≥$60,000	203 (40.2%)	206 (40.8%)	55 (10.9%)	41 (8.1%)	<0.01
	$40,000-<$60,000	54 (27.8%)	84 (43.3%)	37 (19.1%)	19 (9.8%)	
	$25,000-<$40,000	35 (27.3%)	64 (50.0%)	21 (16.4%)	8 (6.3%)	
	<$25,000	6 (20.7%)	10 (34.5%)	7 (24.1%)	6 (20.7%)	
	Missing	36 (21.1%)	73 (42.7%)	33 (19.3%)	29 (17.0%)	<0.01**
Ethnicity	Caucasian	303 (34.3%)	375 (42.4%)	127 (14.4%)	79 (8.9%)	<0.01
	Other	5 (12.8%)	18 (46.2%)	6 (15.4%)	10 (25.6%)	
	Missing	26 (25.0%)	44 (42.3%)	20 (19.2%)	14 (13.5%)	0.57
Emotional distress	(Continuous)	29.0 (9.2)	28.7 (8.6)	29.4 (10.6)	29.5 (9.0)	0.84
	Nonmissing	288 (36.0%)	335 (41.9%)	111 (13.9%)	66 (8.3%)	
	Missing	46 (20.3%)	102 (44.9%)	42 (18.5%)	36 (16.3%)	<0.01**

Variable	Level	≥75 nmol/L n=334	50- <75 nmol/L n=437	37.5- <50 nmol/L n=153	<37.5 nmol/L n=103	P-value
Season	Late summer/ early fall	142 (57.5%)	82 (33.2%)	19 (7.7%)	4 (1.6%)	<0.01
	Late spring/ early summer	113 (35.3%)	140 (43.8%)	48 (15.0%)	19 (5.9%)	
	Late fall/ early winter	45 (21.1%)	102 (47.9%)	39 (18.3%)	27 (12.7%)	
	Late winter/ early spring	34 (13.8%)	113 (45.8%)	47 (19.0%)	53 (21.5%)	

* n (row %) shown except for continuous variables for which mean [standard deviation (SD)] are shown
**P-values adjacent to the proportion missing are based on the comparison of the percentage of missing data on that variable between 25(OH)D categories

4.3 Preterm Birth

4.3.1 Description of Preterm Study Population

Study population characteristics by case-control status are shown in Table 4.2. There were 222 mother-infant pairs with a PTB infant and 1027 control pairs included in this study after the inclusion and exclusion criteria were applied as described in the inclusion/exclusion section of this paper. Using a p-value of ≤0.10 as a cut-off for determining whether or not a factor was to be considered as a potential confounder, several factors were discovered to be different between case and control groups. Among PTB cases, rates of pre-pregnancy physical activity, physical activity during pregnancy, response rates regarding marital status, and rates of infant season of birth were all different from controls by this criterion. Cases and controls were equally likely to engage in physical activity at least twice a week before pregnancy (72.3% vs. 73.4%); however, mothers of infants with PTB were more likely than controls to be completely inactive (11.7% vs. 6.9%). Hence cases and controls were deemed to have different levels of pre-

pregnancy physical activity (p=0.04). During pregnancy, mothers of infants who were born prematurely had lower rates of physical activity two or more times a week (60.0% vs. 67.5%) and higher rates of complete inactivity (17.1% vs. 12.8%). There was a higher response rate for marital status among cases than among controls for this question (69.8% mothers of cases responded to this question vs. 63.6% mothers of controls, p=0.09). The highest number of PTB cases were born in the late summer/ early fall (32.0% vs. 25.1% for controls), while the highest number of controls were born in the late spring/ early summer (31.2% vs. 26.6% for PTB cases).

Categorized and continuous 25(OH)D concentrations by PTB and control status are displayed in Table 4.3. The proportion of cases and controls did not significantly differ among the categories of 25(OH)D concentrations <37.5, 37.5-≤50, 50-≤75 and >75 nmol/L (p=0.11). The percentage of participants with 25(OH)D levels <75 nmol/L was 59.5% for neonates with PTB and 67.5% for controls. On the other hand, 10.8% of PTB and 10.0% of control cord blood samples had 25(OH)D concentrations <37.5 nmol/L. Mean vitamin D concentrations were marginally different between cases with PTB and controls (69.8 ± 25.5 nmol/L vs. 66.5 ± 23.7 nmol/L; p=0.06).

4.3.2 Association between Cord Blood 25(OH)D Concentration and Preterm Birth (Complete Case Analysis)

Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and PTB are displayed in Table 4.4. Complete data for the factors included as confounders (categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress) was available for 61.7% of

the PTB cases and 65.9% of the controls. When the analysis was limited to cases and controls with complete covariate information none of the crude ORs comparing 25(OH)D concentrations below 37.5 nmol/L, 37.5- <50 nmol/L, or 50-<75 nmol/L to 25(OH)D concentrations ≥75 nmol/L reached statistical significance. The ORs comparing risk of PTB based on 25(OH)D concentrations <37.5 vs. ≥37.5 nmol/L, <50 vs. ≥50 nmol/L, and <75 vs. ≥75 nmol/L also failed to reach statistical significance. Variables were included as confounders if they made at least a 5% difference to any OR for any neonatal outcome. Adjusting for confounders did not have a major impact on the association between cord blood 25(OH)D concentration and PTB. The logistic regression adjusting for confounders did not reveal any statistically significant ORs for the relationship between 25(OH)D concentration group and risk of PTB.

4.3.3 Association between Cord Blood 25(OH)D Concentration and Preterm Birth (Multiple Imputation Analysis)

Using multiple imputation to assign missing covariate information based on other non-missing covariate information provided for each mother-baby pair allowed for the inclusion of all 222 PTB cases and 1027 controls. Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and PTB using the imputed values for missing data are shown in Table 4.5. The results from this analysis suggest a slightly protective association of 25(OH)D concentrations between 50-<75 nmol/L compared to levels ≥75 nmol/L with PTB (OR 0.69; 95% CI 0.49-0.96). A similar finding was discovered in the examination of risk of PTB based on levels <75 vs. ≥75 nmol/L (OR 0.71; 95% CI 0.53-0.95).

Adjusting for confounders did not alter the direction or statistical significance of any ORs. Having 25(OH)D concentrations 50-<75nmol/L was associated with reduced odds for PTB relative to 25(OH)D ≥75 nmol/L (aOR 0.67; 95% CI 0.47-0.94). Vitamin D concentrations <75 vs. ≥75 nmol/L were associated with reduced odds for PTB (aOR 0.68; 95% CI 0.50-0.92).

4.3.4 Interactions

Interactions between vitamin D status and maternal BMI, smoking and age were explored to see if any of these three factors modified the association between Vitamin D status and risk of PTB. As the significance of the interaction terms between vitamin D status and maternal BMI, smoking and age were all p >0.10 (p=0.26, 0.97, 0.67, respectively), these interaction terms were not explored further and were not included in the final models.

4.3.5 Tables

Table 4.2: Study Population Characteristics by Preterm Birth Case-Control Status*

Variable	Level	Cases with PTB n = 222	Controls n = 1027	P-value
Age	(Continuous)	29.3 ± 4.3	29.5 ± 4.2	0.36
Categorized age	<25 years old 25-<35 years old ≥35 years old	31 (14.0%) 163 (73.4%) 28 (18.2%)	120 (11.7%) 781 (76.1%) 126 (12.3%)	0.62
BMI	(Continuous) Missing	24.7 ± 6.1 20	24.3 ± 5.3 76	0.41 0.50**
Categorized BMI	<25 kg/m^2 25-<30 kg/m^2 ≥30 kg/m^2 Missing	126 (62.4%) 48 (23.8%) 28 (13.9%) 20	639 (67.2%) 187 (19.7%) 125 (13.1%) 76	0.36 0.50**
Smoking	Not during pregnancy During pregnancy Missing	160 (78.4%) 44 (21.6%) 18	754 (80.9%) 178 (19.1%) 95	0.48 0.68**

Variable	Level	Cases with PTB n = 222	Controls n = 1027	P-value
Second-hand smoke exposure	No Yes Missing	127 (61.7%) 79 (38.4%) 16	619 (64.6%) 340 (35.5%) 68	0.48 0.94**
Physical activity pre-pregnancy	2 or more times/ week 1-3 times/month Not once Missing	149 (72.3%) 33 (16.0%) 24 (11.7%) 16	706 (73.4%) 190 (19.8%) 66 (6.9%) 65	0.04 0.74**
Physical activity during pregnancy	2 or more times/ week 1-3 times/month Not once Missing	123 (60.0%) 47 (22.9%) 35 (17.1%) 17	649 (67.5%) 189 (19.7%) 123 (12.8%) 66	0.10 0.60**
Marital Status	Married Common law Single or separated/divorced Missing	30 (19.4%) 114 (73.6%) 11 (7.1%) 67	157 (24.0%) 460 (70.4%) 36 (5.5%) 374	0.39 0.09**
Education attainment	University CEGEP High school or did not complete high school Missing	74 (38.5%) 68 (35.4%) 50 (26.0%) 30	379 (41.0%) 337 (36.5%) 208 (22.5%) 103	0.56 0.16**
Family income	≥$60,000 $40,000-<60,000 $25,000-<40,000 <$25,000 Missing	108 (61.02%) 36 (20.3%) 24 (13.6%) 9 (5.1%) 45	505 (59.0%) 194 (22.7%) 128 (15.0%) 29 (3.4%) 171	0.62 0.23**
Ethnicity	Caucasian Other Missing	187 (96.4%) 7 (3.6%) 28	884 (95.8%) 39 (4.2%) 104	0.85 0.33**
Emotional distress	(Continuous) Missing	28.3 ± 7.9 45	29.0 ± 9.1 227	0.36 0.61**
Season	Late summer/ early fall Late spring/ early summer Late fall/ early winter Late winter/ early spring	71 (32.0%) 59 (26.6%) 44 (19.8%) 48 (21.6%)	247 (25.1%) 320 (31.2%) 213 (20.7%) 247 (24.1%)	0.10

* n (row %) shown except for continuous variables for which mean (SD) are shown
**P-values adjacent to the proportion missing are based on the comparison of the percentage of missing data on that variable between 25(OH)D categories

Table 4.3: Categorized and Continuous 25(OH)D Concentration by Preterm Birth and Control Status*

Variable	[25(OH)D] (nmol/L)	PTB Cases n=222	Controls n=1027	P-value
Categorized 25(OH)D	<37.5 37.5-<50 50-<75 ≥75	24 (10.8%) 27 (12.2%) 81 (36.5%) 90 (40.5%)	103 (10.0%) 153 (14.9%) 437 (42.6%) 334 (32.5%)	0.11
Continuous 25(OH)D	(Continuous)	69.8 ± 25.4	66.5 ± 23.7	0.06

* n (row %) shown except for continuous variables for which mean (SD) are shown

Table 4.4: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Preterm Birth (Complete Case Analysis)

[25(OH)D] (nmol/L)	PTB Cases n =137 n (%)	Controls n =677 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5 37.5-<50 50-<75 ≥75	11 (8.0%) 15 (11.0%) 51 (37.2%) 60 (43.8%)	50 (7.4%) 87 (12.9%) 283 (41.8%) 257 (38.0%)	0.94 (0.46, 1.92) 0.74 (0.40, 1.37) 0.77 (0.51, 1.16) Ref.	0.81 (0.39, 1.70) 0.71 (0.38, 1.33) 0.74 (0.48, 1.12) Ref.
<37.5 ≥37.5	11 (8.0%) 126 (92.0%)	50 (7.4%) 627 (92.6%)	1.10 (0.56, 2.16) Ref.	0.97 (0.48, 1.97) Ref.
<50 ≥50	26 (19.0%) 111 (81.0%)	137 (20.2%) 540 (79.8%)	0.92 (0.58, 1.47) Ref.	0.87 (0.54, 1.41) Ref.
<75 ≥75	77 (56.2%) 60 (43.8%)	420 (62.0%) 257 (38.0%)	0.79 (0.54, 1.14) Ref.	0.74 (0.51, 1.09) Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

Table 4.5: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Preterm Birth (Multiple Imputation Analysis)

[25(OH)D] (nmol/L)	PTB Cases n =222 n (%)	Controls n =1027 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5 37.5-<50 50-<75 ≥75	24 (10.8%) 27 (12.2%) 81 (36.5%) 90 (40.5%)	103 (10.0%) 153 (14.9%) 437 (42.6%) 334 (32.5%)	0.87 (0.52, 1.43) 0.66 (0.41, 1.05) 0.69 (0.49, 0.96) Ref.	0.80 (0.48, 1.34) 0.62 (0.38, 1.01) 0.67 (0.47, 0.94) Ref.
<37.5 ≥37.5	24 (10.8%) 198 (89.2%)	103 (10.0%) 924 (90.0%)	1.09 (0.68, 1.70) Ref.	1.03 (0.64, 1.68) Ref.
<50 ≥50	51 (23.0%) 171 (77.0%)	256 (24.9%) 771 (75.1%)	0.90 (0.64, 1.27) Ref.	0.86 (0.60, 1.22) Ref.

<75	132 (59.5%)	693 (67.5%)	0.71 (0.53, 0.95)	0.68 (0.50, 0.92)
≥75	90 (40.5%)	334 (32.5%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

4.4 Low Birthweight

4.4.1 Description of Low Birthweight Study Population

Study population characteristics by LBW case-control status are shown in Table 4.6. There were 106 mother-infant pairs with LBW and 1027 control pairs included in this study after the inclusion and exclusion criteria were applied as described Section 3.3.1. Using a p-value of ≤0.10 as a cut-off for determining whether or not a factor was to be considered as a potential confounder, a few factors were found to meet the criteria for potential confounding and several factors were found to differ in terms of 'missingness'. Mothers of LBW infants had lower mean pre-pregnancy BMI than controls (22.7 ± 5.4 kg/m^2 in cases vs. 24.3 ± 5.3 kg/m^2 in controls; p=<0.01). Mothers of LBW infants also had higher rates of smoking during pregnancy (27.6% vs. 19.1%; p=0.08). A higher proportion of mothers with LBW babies were inactive than control mothers (12.1% vs. 6.9% respectively). Mothers of LBW infants also had a higher frequency of exercising two or more times per week than controls (78.0% vs. 73.4%). Maternal pre-pregnancy physical activity was different between cases and controls (p=0.02).

Controls tended to have more complete covariate information than cases. This pattern was consistent across all covariates with missing data except for marital status and emotional distress. There was more complete information on marital status for cases than for controls (24.5% missing vs. 36.4%; p=0.02). The amount of complete emotional distress data was similar between cases and controls (20.8% vs. 22.1%; p=0.84).

Categorized and continuous 25(OH)D by LBW and control status are displayed in Table 4.7. Groups categorized based on 25(OH)D concentrations <37.5, 37.5-≤50, 50-≤75 and >75 nmol/L were different between cases and controls (p=0.03). Cases had fewer cord blood samples with 25(OH)D concentrations <75 nmol/L than controls (53.8% vs. 67.5%). Cases and controls had the same proportion of cord blood samples with 25(OH)D concentrations <37.5 nmol/L (9.4% vs. 10.0%). Mean vitamin D status was higher among cases with LBW than controls (72.4 ± 25.9 nmol/L vs. 66.5 ± 23.7 nmol/L; p=0.01).

4.4.2 Association Between Cord Blood 25(OH)D Concentration and Low Birthweight (Complete Case Analysis)

Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and LBW are displayed in Table 4.8. Among cases with LBW, 58.5% had complete data for the factors included as confounders (categorical BMI, physical activity before and during pregnancy, education, family income, and emotional distress). Among controls the percentage of participants with full information on all of these confounders was 65.9%. When the analysis was limited to cases and controls with complete covariate information none of the crude ORs comparing 25(OH)D concentrations below 37.5 nmol/L, 37.5- <50 nmol/L, or 50-<75 nmol/L to 25(OH)D concentrations ≥75 nmol/L had statistically significant ORs. Adjusting for confounders did not have a major impact on any of these associations. The ORs comparing risk of LBW based on 25(OH)D concentrations <37.5 vs. ≥37.5 nmol/L, <50 vs. ≥50 nmol/L, and <75 vs. ≥75 nmol/L revealed a slightly protective association between 25(OH)D concentrations <75 nmol/L compared to ≥75 nmol/L (OR 0.54; 95% CI 0.32-0.91). Adjusting for confounders

widened the confidence interval slightly, but did not change the point estimate (aOR 0.54; 95% CI 0.32-0.94).

4.4.3 Association Between Cord Blood 25(OH)D Concentration and Low Birthweight (Multiple Imputation Analysis)

Using multiple imputation to assign missing covariate information based on the covariate information provided for each mother-baby pair allowed for the inclusion of all 106 LBW cases and 1027 controls. Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and LBW using the imputed values for missing data are shown in Table 4.9. The results from this analysis suggest a slightly protective influence of having vitamin D status between 37.5-<50 nmol/L vs. ≥75 nmol/L (OR 0.45, 95% CI 0.22-0.90). The relationship between having vitamin D status between 50-<75 nmol/L vs. ≥75 nmol/L and risk of LBW was also in a protective direction (OR 0.58, 95% CI 0.37-0.91). Having 25(OH)D concentrations <75 nmol/L vs. ≥75 nmol/L had a protective association with risk of LBW (OR 0.56, 95% CI 0.37-0.84).

These associations were maintained after adjusting for confounders. The association between 25(OH)D concentrations 37.5-<50 nmol/L vs. ≥75 nmol/L was strengthened by the inclusion of confounders (aOR 0.40, 95% CI 0.19-0.82). The association between 25(OH)D concentrations 50-<75 nmol/L vs. ≥75 nmol/L was also strengthened after adjusting for confounders (aOR 0.53, 95% CI 0.34-0.85), as was the association between 25(OH)D concentrations <75 nmol/L vs. ≥75 nmol/L (aOR 0.51, 95% CI 0.34-0.78).

4.4.4 Interactions

Interactions between vitamin D status and maternal BMI, smoking and age were explored to see if any of these three factors modified the association between vitamin D status and risk of LBW. None of the factors examined modified the association between vitamin D status and risk of LBW. As the significance of the interaction terms between vitamin D status and maternal BMI, smoking and age were all >0.10 (p=0.99, 0.43, 0.99, respectively), these interaction terms were not explored further and were not included in the final models.

4.4.5 Tables

Table 4.6: Study Population Characteristics by Low Birthweight Case-Control Status*

Variable	Level	Cases with LBW n = 106	Controls n = 1027	P-value
Age	(Continuous)	29.4 ± 4.2	29.5 ± 4.2	0.72
Categorized age	<25 years old 25-<35 years old ≥35 years old	12 (11.3%) 82 (77.4%) 12 (11.3%)	120 (11.7%) 781 (76.1%) 126 (12.3%)	0.95
BMI	(Continuous) Missing	22.7 ± 5.4 18	24.3 ± 5.3 76	<0.01 <0.01**
Categorized BMI	<25 kg/m^2 25-<30 kg/m^2 ≥30 kg/m^2 Missing	66 (75.0%) 13 (14.8%) 9 (10.2%) 18	639 (67.2%) 187 (19.7%) 125 (13.1%) 76	0.32 <0.01**
Smoking	Not during pregnancy During pregnancy Missing	63 (72.4%) 24 (27.6%) 19	754 (80.9%) 178 (19.1%) 95	0.08 <0.01**
Second-hand smoke exposure	No Yes Missing	55 (61.1%) 35 (38.9%) 16	619 (64.6%) 340 (35.5%) 68	0.59 <0.01**
Physical activity pre-pregnancy	2 or more times/ week 1-3 times/month Not once Missing	71 (78.0%) 9 (9.9%) 11 (12.1%) 15	706 (73.4%) 190 (19.8%) 66 (6.9%) 65	0.02 <0.01**

Variable	Level	Cases with LBW n = 106	Controls n = 1027	P-value
Physical activity during pregnancy	2 or more times/ week 1-3 times/month Not once Missing	59 (64.8%) 19 (20.9%) 13 (14.3%) 15	649 (67.5%) 189 (19.7%) 123 (12.8%) 66	0.86 <0.01**
Marital Status	Married Common law Single or separated/divorced Missing	15 (18.8%) 60 (75.0%) 5 (6.3%) 26	157 (24.0%) 460 (70.4%) 36 (5.5%) 374	0.57 0.02**
Education attainment	University CEGEP High school or did not complete high school Missing	28 (32.6%) 34 (39.5%) 24 (27.9%) 20	379 (41.0%) 337 (36.5%) 208 (22.5%) 103	0.27 <0.01**
Family income	≥$60,000 $40,000-<60,000 $25,000-<40,000 <$25,000 Missing	45 (61.6%) 13 (17.8%) 10 (13.7%) 5 (6.9%) 33	505 (59.0%) 194 (22.7%) 128 (15.0%) 29 (3.4%) 171	0.38 <0.01**
Ethnicity	Caucasian Other Missing	84 (94.4%) 2 (2.3%) 20	884 (%) 39 (%) 104	0.57 <0.01**
Emotional distress	(Continuous) Missing	28.0 ± 7.6 22	29.0 ± 9.1 227	0.29 0.84**
Season	Late summer/ early fall Late spring/ early summer Late fall/ early winter Late winter/ early spring	30 (28.3%) 30 (28.3%) 26 (24.5%) 20 (18.9%)	247 (24.1%) 320 (31.2%) 213 (20.7%) 247 (24.1%)	0.44

* n (row %) shown except for continuous variables for which mean (SD) are shown
**P-values adjacent to the proportion missing are based on the comparison of the percentage of missing data on that variable between 25(OH)D categories

Table 4.7: Categorized and Continuous 25(OH)D Concentration by Low Birthweight and Control Status*

Variable	[25(OH)D] (nmol/L)	LBW Cases n=106	Controls n=1027	P-value
Categorized 25(OH)D	<37.5 37.5-<50 50-<75 ≥75	10 (9.4%) 10 (9.4%) 37 (34.9%) 49 (46.2%)	103 (10.0%) 153 (14.9%) 437 (42.6%) 334 (32.5%)	0.03
Continuous 25(OH)D	(Continuous)	72.4 ± 25.9	66.5 ± 23.7	0.01

* n (row %) shown except for continuous variables for which mean (SD) are shown

Table 4.8: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Low Birthweight (Complete Case Analysis)

[25(OH)D] (nmol/L)	LBW Cases n = 62 n (%)	Controls n =677 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5	2 (3.2%)	50 (7.4%)	0.31 (0.07, 1.34)	0.30 (0.07, 1.36)
37.5-<50	6 (9.7%)	87 (12.9%)	0.54 (0.22, 1.33)	0.54 (0.22, 1.37)
50-<75	21 (33.9%)	283 (41.8%)	0.58 (0.33, 1.03)	0.58 (0.33, 1.05)
≥75	33 (53.2%)	257 (38.0%)	Ref.	Ref.
<37.5	2 (3.2%)	50 (7.4%)	0.42 (0.10, 1.76)	0.47 (0.11, 2.07)
≥37.5	60 (96.8%)	627 (92.6%)	Ref.	Ref.
<50	8 (12.9%)	137 (20.2%)	0.58 (0.27, 1.26)	0.63 (0.29, 1.39)
≥50	54 (87.1%)	540 (79.8%)	Ref.	Ref.
<75	29 (46.8%)	420 (62.0%)	0.54 (0.32, 0.91)	0.54 (0.32, 0.94)
≥75	33 (53.2%)	257 (38.0%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, and emotional distress

Table 4.9: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Low Birthweight (Multiple Imputation Analysis)

[25(OH)D] (nmol/L)	LBW Cases n =106 n (%)	Controls n =1027 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5	10 (9.4%)	103 (10.0%)	0.66 (0.32, 1.35)	0.59 (0.28, 1.26)
37.5-<50	10 (9.4%)	153 (14.9%)	0.45 (0.22, 0.90)	0.40 (0.19, 0.82)
50-<75	37 (34.9%)	437 (42.6%)	0.58 (0.37, 0.91)	0.53 (0.34, 0.85)
≥75	49 (46.2%)	334 (32.5%)	Ref.	Ref.
<37.5	10 (9.4%)	103 (10.0%)	0.93 (0.47, 1.85)	0.90 (0.44, 1.84)
≥37.5	96 (90.6%)	924 (90.0%)	Ref.	Ref.
<50	20 (18.9%)	256 (24.9%)	0.70 (0.42, 1.16)	0.66 (0.39, 1.11)
≥50	86 (81.1%)	771 (75.1%)	Ref.	Ref.
<75	57 (53.8%)	693 (67.5%)	0.56 (0.37, 0.84)	0.51 (0.34, 0.78)
≥75	49 (46.2%)	334 (32.5%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

4.5 Small for Gestational Age

4.5.1 Description of Small for Gestational Age Study Population

Study population characteristics by case-control status are shown in Table 4.10. There were 301 mother-infant pairs with an SGA infant and 1027 controls pairs included after the inclusion and exclusion criteria were applied. Using a p-value of ≤0.10, many factors were deemed to be potential confounders. Mean pre-pregnancy BMI was lower among cases than controls (23.2 ± 4.9 vs. 24.3 ± 5.3 respectively; $p<0.01$). Although the proportion of mothers with a BMI between 25-<30 kg/m^2 was comparable between cases and controls (19.6% vs. 19.7%), mothers of cases who were SGA were more often of normal or healthy weight (72.6% vs. 67.2% among controls) while mothers of controls were more likely to have BMI ≥30 kg/m^2 (13.1% vs. 7.9% among cases). Cases had lower BMI overall ($p=0.06$). Mothers of infants who were born SGA were more likely to smoke during pregnancy compared to controls (30.5% vs. 19.1%; $p<0.01$). These mothers were also more likely to be exposed to second-hand smoke (41.9% vs. 35.5%; $p=0.06$). Maternal pre-pregnancy physical activity varied by SGA case/control status ($p=0.02$), although maternal physical activity during pregnancy did not differ between these two groups ($p=0.71$). Mothers of cases reported exercising 2 or more times per week more often than mothers of controls (79.0% vs. 73.4%). Cases had lower rates of exercising 1-3 times per month compared to controls (12.6% vs. 19.8%) and a higher rate of complete inactivity (8.5% vs. 6.9%). Mothers of cases were less likely to be married (14.8% for cases vs. 24.0% for controls) and more likely to be living common law (77.5% for cases vs. 70.4% for controls), and had slightly higher rates of being single or separated/divorced (7.7% vs. 5.5%). Driven primarily by the lower rate of marriage

among cases vs. controls, cases had lower rates of marriage and higher rates of being single or separated/divorced than controls (p=0.01). Mothers of SGA infants were less likely to have complete information on all of the covariates with any missing data compared to mothers of controls, with the exception of marital status (30.6% vs. 36.4% among controls; p=0.07), ethnicity (p=0.26) and emotional distress (p=0.15).

Categorized and continuous 25(OH)D by SGA and control status are displayed in Table 4.11. Groups categorized based on 25(OH)D concentrations <37.5, 37.5-≤50, 50-≤75 and >75 nmol/L did not differ between cases and controls (p=0.49). The percentage of participants with 25(OH)D concentrations <75 nmol/L was 65.1% among SGA infants and 67.5% among controls. Mean vitamin D did not differ between cases who were SGA and controls (67.2 ± 25.8 nmol/L vs. 66.5 ± 23.7 nmol/L; p=0.66).

4.5.2 Association Between Cord Blood 25(OH)D Concentration and Small for Gestational Age (Complete Case Analysis)

Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and SGA are displayed in Table 4.12. There was complete information on confounders for 57.5% of cases and 65.9% of controls. Limiting the analysis to cases and controls with complete covariate information, none of the crude ORs comparing 25(OH)D concentrations below 37.5 nmol/L, 37.5- <50 nmol/L, or 50-<75 nmol/L to 25(OH)D concentrations ≥75 nmol/L reached statistical significance. Having 25(OH)D concentrations <37.5 vs. ≥37.5 nmol/L, <50 vs. ≥50 nmol/L, and <75 vs. ≥75 nmol/L was not associated with being born SGA. Adjusting for confounders did not change any of these relationships.

4.5.3 Association between Cord Blood 25(OH)D Concentration and Small for Gestational Age (Multiple Imputation Analysis)

Assigning missing covariate information based on the covariate information included for each mother-baby pair using multiple imputation enabled 301 SGA cases and 1027 to be included in the analysis. Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and SGA including the imputed values for missing data are shown in Table 4.13. None of these associations was statistically significant. The associations did not change appreciably with the inclusion of confounders.

4.5.4 Interactions

Interactions between vitamin D status and maternal BMI, smoking and age were explored to see if any of these three factors modified the association between vitamin D status and risk of SGA. As described in the methods section, effect modification between 25(OH)D and risk of SGA was assessed using adjusted complete case models. Categorical BMI was found to modify the relationship between vitamin D status and risk of SGA ($p=0.05$). Table 4.14 shows the pooled and stratified ORs for the association between cord blood 25(OH)D concentration and SGA status. In examining the association between vitamin D status and risk of SGA stratified by categorical BMI none of the ORs reached statistical significance. The interaction terms between smoking status during pregnancy and vitamin D status as well as categorical age and vitamin D status were not statistically significant ($p=0.51$ and $p=0.66$, respectively). Hence these interaction terms were not explored further.

4.5.5 Tables

Table 4.10: Study Population Characteristics by Small for Gestational Age Case-Control Status*

Variable	Level	Cases with SGA n = 301	Controls n = 1027	P-value
Age	(Continuous)	29.6 (4.2)	29.5 (4.2)	0.81
Categorized age	<25 years old 25-<35 years old ≥35 years old	30 (10.0%) 229 (76.1%) 42 (14.0%)	120 (11.7%) 781 (76.1%) 126 (12.3%)	0.57
BMI	(Continuous) Missing	23.2 (4.9) 35	24.3 (5.3) 76	<0.01 0.03**
Categorized BMI	<25 kg/m^2 25-<30 kg/m^2 ≥30 kg/m^2 Missing	193 (72.6%) 52 (19.6%) 21 (7.9%) 35	639 (67.2%) 187 (19.7%) 125 (13.1%) 76	0.06 0.03**
Smoking	Not during pregnancy During pregnancy Missing	182 (69.5%) 80 (30.5%) 39	754 (80.9%) 178 (19.1%) 95	<0.01 0.08**
Second-hand smoke exposure	No Yes Missing	158 (58.1%) 114 (41.9%) 29	619 (64.6%) 340 (35.5%) 68	0.06 0.09**
Physical activity pre-pregnancy	2 or more times/ week 1-3 times/month Not once Missing	214 (79.0%) 34 (12.6%) 23 (8.5%) 30	706 (73.4%) 190 (19.8%) 66 (6.9%) 65	0.02 0.04**
Physical activity during pregnancy	2 or more times/ week 1-3 times/month Not once Missing	183 (67.5%) 49 (18.1%) 39 (14.4%) 30	649 (67.5%) 189 (19.7%) 123 (12.8%) 66	0.71 0.05**
Marital Status	Married Common law Single or separated/divorced Missing	31 (14.8%) 162 (77.5%) 16 (7.7%) 92	157 (24.0%) 460 (70.4%) 36 (5.5%) 374	0.01 0.07**
Education attainment	University CEGEP High school or did not complete high school Missing	96 (37.2%) 98 (38.0%) 64 (24.8%) 43	379 (41.0%) 337 (36.5%) 208 (22.5%) 103	0.52 <0.05**
Family income	≥$60,000 $40,000-<60,000 $25,000-<40,000 <$25,000 Missing	128 (55.4%) 58 (25.1%) 37 (16.0%) 8 (3.5%) 70	505 (59.0%) 194 (22.7%) 128 (15.0%) 29 (3.4%) 171	0.80 0.01**

Variable	Level	Cases with SGA n = 301	Controls n = 1027	P-value
Ethnicity	Caucasian Other Missing	256 (97.3%) 7 (2.7%) 38	884 (95.8%) 39 (4.2%) 104	0.33 0.26**
Emotional distress	(Continuous) Missing	29.7 (9.0) 79	29.0 (9.1) 227	0.28 0.15**
Season	Late summer/ early fall Late spring/ early summer Late fall/ early winter Late winter/ early spring	70 (23.3%) 88 (29.2%) 77 (25.6%) 66 (21.9%)	247 (24.1%) 320 (31.2%) 213 (20.7%) 247 (24.1%)	0.35

* n (row %) shown except for continuous variables for which mean (SD) are shown
**P-values adjacent to the proportion missing are based on the comparison of the percentage of missing data on that variable between 25(OH)D categories

Table 4.11: Categorized and Continuous 25(OH)D Concentration by Small for Gestational Age and Control Status*

Variable	[25(OH)D] (nmol/L)	SGA Cases n=301	Controls n=1027	P-value
Categorized 25(OH)D	<37.5 37.5-<50 50-<75 ≥75	37 (12.3%) 40 (13.3%) 119 (39.5%) 105 (34.9%)	103 (10.0%) 153 (14.9%) 437 (42.6%) 334 (32.5%)	0.49
Continuous 25(OH)D	(Continuous)	67.2 ± 25.8	66.5 ± 23.7	0.66

* n (row %) shown except for continuous variables for which mean (SD) are shown

Table 4.12: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Small for Gestational Age (Complete Case Analysis)

[25(OH)D] (nmol/L)	SGA Cases n = 173 n (%)	Controls n = 677 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5	13 (7.5%)	50 (7.4%)	0.88 (0.45, 1.70)	0.92 (0.46, 1.83)
37.5-<50	16 (9.3%)	87 (12.9%)	0.62 (0.34, 1.12)	0.64 (0.35, 1.17)
50-<75	68 (39.3%)	283 (41.8%)	0.81 (0.56, 1.17)	0.79 (0.54, 1.15)
≥75	76 (43.9%)	257 (38.0%)	Ref.	Ref.
<37.5	13 (7.5%)	50 (7.4%)	1.02 (0.54, 1.92)	1.08 (0.56, 2.09)
≥37.5	160 (92.5%)	627 (92.6%)	Ref.	Ref.
<50	29 (16.8%)	137 (20.2%)	0.79 (0.51, 1.23)	0.83 (0.53, 1.31)
≥50	144 (83.2%)	540 (79.8%)	Ref.	Ref.
<75	97 (56.1%)	420 (62.0%)	0.78 (0.56, 1.11)	0.77 (0.54, 1.11)
≥75	76 (43.9%)	257 (38.0%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

Table 4.13: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Small for Gestational Age (Multiple Imputation Analysis)

[25(OH)D] (nmol/L)	SGA Cases n =301 n (%)	Controls n =1027 n (%)	Crude OR (95% CI)	Adjusted‡ OR (95% CI)
<37.5	37 (12.3%)	103 (10.0%)	1.14 (0.74, 1.77)	1.18 (0.75, 1.86)
37.5-<50	40 (13.3%)	153 (14.9%)	0.83 (0.55, 1.26)	0.80 (0.52, 1.22)
50-<75	119 (39.5%)	437 (42.6%)	0.87 (0.64, 1.17)	0.86 (0.63, 1.16)
≥75	105 (34.9%)	334 (32.5%)	Ref.	Ref.
<37.5	37 (12.3%)	103 (10.0%)	1.26 (0.84, 1.88)	1.32 (0.88, 2.00)
≥37.5	264 (87.7%)	924 (90.0%)	Ref.	Ref.
<50	77 (25.6%)	256 (24.9%)	1.04 (0.77, 1.39)	1.03 (0.76, 1.40)
≥50	224 (74.4%)	771 (75.1%)	Ref.	Ref.
<75	196 (65.1%)	693 (67.5%)	0.90 (0.67, 1.18)	0.89 (0.67, 1.17)
≥75	105 (34.9%)	334 (32.5%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

Table 4.14: Crude Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Small for Gestational Age (Pooled and Stratified by BMI, Complete Case Analysis)

BMI (kg/m^2)	[25(OH)D] (nmol/L)	SGA Cases n (%)	Controls n (%)	OR (95% CI)
All	<37.5	31 (25.0%)	93 (75.0%)	1.14 (0.71, 1.82)
	37.5-<50	36 (20.7%)	138 (79.3%)	0.89 (0.58, 1.37)
	50-<75	107 (20.9%)	406 (79.1%)	0.90 (0.66, 1.23)
	≥75	92 (22.7%)	314 (77.3%)	Ref.
<25	<37.5	21 (10.9%)	55 (8.6%)	1.29 (0.73, 2.28)
	37.5-<50	20 (10.4%)	88 (13.8%)	0.77 (0.44, 1.34)
	50-<75	84 (43.5%)	267 (41.8%)	1.06 (0.74, 1.53)
	≥75	68 (35.2%)	229 (35.8%)	Ref.
25-<30	<37.5	6 (11.5%)	25 (13.4%)	0.68 (0.25, 1.91)
	37.5-<50	8 (15.4%)	29 (15.5%)	0.79 (0.31, 2.00)
	50-<75	18 (34.6%)	76 (40.6%)	0.68 (0.33, 1.39)
	≥75	20 (38.5)	57 (30.5%)	Ref.
≥30	<37.5	4 (19.1%)	13 (10.4%)	2.15 (0.46, 9.99)
	37.5-<50	8 (38.1%)	21 (16.8%)	2.67 (0.71, 10.05)
	50-<75	5 (23.8%)	63 (50.4%)	0.56 (0.14, 2.23)
	≥75	4 (19.1%)	28 (22.4%)	Ref.

4.6 Adverse Neonatal Outcomes

4.6.1 Description of Full Study Population

Study population characteristics by composite case-control status are shown in Table 4.15. There were 517 mother-infants pairs with a PTB, LBW and/or SGA infant and 1027 control pairs included after applying the inclusion and exclusion criteria. Several factors were considered potential confounders based on a ≤0.10 p-value cut-off. Mothers in the case group were more likely to smoke during pregnancy than mothers in the control group (26.0% vs. 19.1%; p<0.01). Exposure to second-hand smoke during pregnancy was also higher among cases than controls (40.1% vs. 35.5%; p=0.09). Pre-pregnancy physical activity was different among mothers of cases than among mothers of controls (p=0.01), with mothers of cases having higher rates of physical activity two or more times per week (76.1% vs. 73.4%) but also greater rates of complete inactivity (9.7% vs. 6.9%). Fewer cases were married than controls (p=0.02), with rates of marriage at 16.7% among cases (vs. 24.0% among controls), balanced out mainly by cohabitation (75.8% for cases vs. 70.4% for controls) and also by slightly higher rates of being single or separated/divorced (7.5% vs. 5.5%). Cases were less likely to report their BMI (10.3% missing vs. 7.4%; p=0.06), education attainment (13.5% missing vs. 10.0%; p=0.04) and family income (21.9% missing vs. 16.7%; p=0.01). Controls were less likely to report their marital status (36.4% missing among controls vs. 30.4% among cases; p=0.02).

Categorized and continuous 25(OH)D by case and control status are displayed in Table 4.16. Vitamin D groups categorized as <37.5, 37.5-≤50, 50-≤75 and >75 nmol/L did not differ between cases and controls (p=0.24). Among cases, 63.1% of umbilical cord samples had vitamin D concentrations <75 nmol/L. Among controls, 67.5% of

samples had vitamin D concentrations below this cut-point. Mean vitamin D did not differ by overall case/ control status (68.1 ± 25.5 vs. 66.5 ± 23.7; p=0.24).

4.6.2 Association Between Cord Blood 25(OH)D Concentration and Case Status (Complete Case Analysis)

Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and case status are displayed in Table 4.17. There was complete information on confounders for 59.2% of cases and 65.9% of controls. Limiting the analysis to cases and controls with complete covariate information, none of the crude ORs comparing 25(OH)D concentrations below 37.5, 37.5- <50, or 50-<75 nmol/L to 25(OH)D concentrations ≥75 nmol/L reached statistical significance. Having 25(OH)D concentrations <37.5 vs. ≥37.5, <50 vs. ≥50, and <75 vs. ≥75 nmol/L was not associated with greater risk of PTB, LBW, and/or SGA. Adjusting for confounders revealed an association between 25(OH)D concentrations <75 nmol/L vs. ≥75 nmol/L (aOR 0.75, 95% CI 0.57-1.00).

4.6.3 Association Between Cord Blood 25(OH)D Concentration and Case Status (Multiple Imputation Analysis)

After imputing for missing covariate information based on the complete data provided, 517 cases and 1027 controls were included for analysis. Crude and adjusted ORs for the association between cord blood 25(OH)D concentration and case status using multiple imputation are shown in Table 4.18. After adjusting for confounders, 25(OH)D concentrations 50-<75 nmol/L showed lower odds of the composite case status compared to concentrations ≥75 nmol/L (aOR 0.77; 95% CI 0.60, 0.99).

4.6.4 Interactions

Categorical BMI was found to modify the relationship between vitamin D status and risk of PTB, LBW and/or SGA (p=0.07). Table 4.19 shows the pooled and stratified ORs for the association between cord blood 25(OH)D concentration and case status. Babies of mothers with a BMI ≥30 who had umbilical cord 25(OH)D concentrations between 50-<75 nmol/L had lower odds of having PTB, LBW and/or SGA than those with 25(OH)D concentrations ≥75 nmol/L (OR 0.42, 95% CI 0.18-0.97). Neither smoking status during pregnancy nor categorical age modified the relationship between vitamin D and the risk of case status in adjusted models (p=0.79 and p=0.57, respectively). Hence these were not explored further.

4.6.5 Tables

Table 4.15: Study Population Characteristics by Composite Case-Control Status*

Variable	Level	Cases n = 517	Controls n = 1027	P-value
Age	(Continuous)	29.4 ± 4.4	29.5 ± 4.2	0.57
Categorized age	<25 years old 25-<35 years old ≥35 years old	61 (11.8%) 388 (75.1%) 68 (13.2%)	120 (11.7%) 781 (76.1%) 126 (12.3%)	0.88
BMI	(Continuous) Missing	23.8 ± 5.5 53	24.3 ± 5.3 76	0.11 0.06**
Categorized BMI	<25 kg/m^2 25-<30 kg/m^2 ≥30 kg/m^2 Missing	319 (68.8%) 95 (20.5%) 50 (10.8%) 53	639 (67.2%) 187 (19.7%) 125 (13.1%) 76	0.44 0.06**
Smoking	Not during pregnancy During pregnancy Missing	342 (74.0%) 120 (26.0%) 55	754 (80.9%) 178 (19.1%) 95	<0.01 0.39**
Second-hand smoke exposure	No Yes Missing	284 (59.9%) 190 (40.1%) 43	619 (64.6%) 340 (35.5%) 68	0.09 0.23**

Variable	Level	Cases n = 517	Controls n = 1027	P-value
Physical activity pre-pregnancy	2 or more times/ week 1-3 times/month Not once Missing	360 (76.1%) 67 (14.2%) 46 (9.7%) 44	706 (73.4%) 190 (19.8%) 66 (6.9%) 65	0.01 0.12**
Physical activity during pregnancy	2 or more times/ week 1-3 times/month Not once Missing	304 (64.4%) 94 (19.9%) 74 (15.7%) 45	649 (67.5%) 189 (19.7%) 123 (12.8%) 66	0.30 0.11**
Marital Status	Married Common law Single or separated/divorced Missing	60 (16.7%) 273 (75.8%) 27 (7.5%) 157	157 (24.0%) 460 (70.4%) 36 (5.5%) 374	0.02 0.02**
Education attainment	University CEGEP High school or did not complete high school Missing	168 (37.6%) 164 (36.7%) 115 (25.7%) 70	379 (41.0%) 337 (36.5%) 208 (22.5%) 103	0.33 0.04**
Family income	≥$60,000 $40,000-<60,000 $25,000-<40,000 <$25,000 Missing	232 (57.4%) 93 (23.0%) 63 (15.6%) 16 (4.0%) 113	505 (59.0%) 194 (22.7%) 128 (15.0%) 29 (3.4%) 171	0.93 0.01**
Ethnicity	Caucasian Other Missing	440 (96.9%) 14 (3.1%) 63	884 (95.8%) 39 (4.2%) 104	0.57 0.22**
Emotional distress	(Continuous) Missing	29.1 ± 8.6 122	29.0 ± 9.1 227	0.80 0.51**
Season	Late summer/ early fall Late spring/ early summer Late fall/ early winter Late winter/ early spring	137 (26.5%) 146 (28.2%) 120 (23.2%) 114 (31.6%)	247 (25.1%) 320 (31.2%) 213 (20.7%) 247 (24.1%)	0.34

* n (row %) shown except for continuous variables for which mean (SD) are shown
**P-values adjacent to the proportion missing are based on the comparison of the percentage of missing data on that variable between 25(OH)D categories

Table 4.16: Categorized and Continuous 25(OH)D Concentration by Composite Case and Control Status*

Variable	[25(OH)D] (nmol/L)	Cases n=517	Controls n=1027	P-value
Categorized 25(OH)D	<37.5 37.5-<50	60 (11.6%) 67 (13.0%)	103 (10.0%) 153 (14.9%)	0.17

	50-<75 ≥75	199 (38.5%) 191 (36.9%)	437 (42.6%) 334 (32.5%)	
Continuous 25(OH)D	(Continuous)	68.1 ± 25.5	66.5 ± 23.7	0.24

* n (row %) shown except for continuous variables for which mean (SD) are shown

Table 4.17: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Composite Case Status (Complete Case Analysis)

[25(OH)D] (nmol/L)	**Cases n =306 n (%)**	**Controls n =677 n (%)**	**Crude OR (95% CI)**	**Adjusted‡ OR (95% CI)**
<37.5	24 (7.8%)	50 (7.4%)	0.92 (0.54, 1.56)	0.89 (0.51, 1.54)
37.5-<50	31 (10.1%)	87 (12.9%)	0.68 (0.43, 1.08)	0.68 (0.42, 1.09)
50-<75	117 (38.2%)	283 (41.8%)	0.79 (0.59, 1.07)	0.75 (0.55, 1.02)
≥75	134 (43.8%)	257 (38.0%)	Ref.	Ref.
<37.5	24 (7.8%)	50 (7.4%)	1.07 (0.64, 1.77)	1.06 (0.63, 1.79)
≥37.5	282 (92.2%)	627 (92.6%)	Ref.	Ref.
<50	55 (18.0%)	137 (20.2%)	0.86 (0.61, 1.22)	0.87 (0.61, 1.24)
≥50	251 (82.0%)	540 (79.8%)	Ref.	Ref.
<75	172 (56.2%)	420 (62.0%)	0.79 (0.60, 1.03)	0.75 (0.57, 1.00)
≥75	134 (43.8%)	257 (38.0%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

Table 4.18: Crude and Adjusted Odds Ratios for the Association Between Cord Blood 25(OH)D Concentration and Composite Case Status (Multiple Imputation Analysis)

[25(OH)D] (nmol/L)	**Cases n = 517 n (%)**	**Controls n =1027 n (%)**	**Crude OR (95% CI)**	**Adjusted‡ OR (95% CI)**
<37.5	60 (11.6%)	103 (10.0%)	1.02 (0.71, 1.47)	1.01 (0.69, 1.47)
37.5-<50	67 (13.0%)	153 (14.9%)	0.77 (0.55, 1.07)	0.72 (0.51, 1.02)
50-<75	199 (38.5%)	437 (42.6%)	0.80 (0.62, 1.02)	0.77 (0.60, 0.99)
≥75	191 (36.9%)	334 (32.5%)	Ref.	Ref.
<37.5	60 (11.6%)	103 (10.0%)	1.18 (0.84, 1.65)	1.20 (0.85, 1.69)
≥37.5	457 (88.4%)	924 (90.0%)	Ref.	Ref.
<50	127 (24.6%)	256 (24.9%)	0.98 (0.77, 1.25)	0.96 (0.75, 1.24)
≥50	390 (75.4%)	771 (75.1%)	Ref.	Ref.
<75	326 (63.1%)	693 (67.5%)	0.82 (0.66, 1.03)	0.79 (0.63, 1.00)
≥75	191 (36.9%)	334 (32.5%)	Ref.	Ref.

‡ Adjusted for categorical BMI, physical activity before and during pregnancy, education, family income, ethnicity, and emotional distress

Table 4.19: Crude Odds Ratios for the Association between Cord Blood 25(OH)D Concentration and Composite Case Status (Pooled and Stratified by BMI, Complete Case Analysis)

BMI (kg/m^2)	[25(OH)D] (nmol/L)	Cases n (%)	Controls n (%)	OR (95% CI)
All	<37.5	49 (10.6%)	93 (9.8%)	0.96 (0.65, 1.42)
	37.5-<50	61 (13.2%)	138 (14.5%)	0.80 (0.56, 1.14)
	50-<75	181 (39.0%)	406 (69.2%)	0.81 (0.63, 1.04)
	≥75	173 (37.3%)	314 (33.0%)	Ref.
<25	<37.5	28 (8.8%)	55 (8.6%)	0.95 (0.57, 1.57)
	37.5-<50	34 (10.7%)	88 (13.8%)	0.72 (0.46, 1.13)
	50-<75	134 (42.0%)	267 (41.8%)	0.93 (0.69, 1.26)
	≥75	123 (38.6%)	229 (35.8%)	Ref.
25-<30	<37.5	13 (13.7%)	25 (13.4%)	0.85 (0.38, 1.87)
	37.5-<50	14 (14.7%)	29 (15.5%)	0.79 (0.37, 1.69)
	50-<75	33 (34.7%)	76 (40.6%)	0.71 (0.39, 1.27)
	≥75	35 (36.8%)	57 (30.5%)	Ref.
≥30	<37.5	8 (16.0%)	13 (10.4%)	1.15 (0.39, 3.39)
	37.5-<50	13 (26.0%)	21 (16.8%)	1.16 (0.45, 2.94)
	50-<75	14 (28.0%)	63 (50.4%)	0.42 (0.18, 0.97)
	≥75	15 (30.0%)	28 (22.4%)	Ref.

4.7 Secondary Objective: Relationship between Maternal Vitamin D and Neonatal Vitamin D

The relationship between maternal and neonatal 25(OH)D is illustrated in Figure 4.1. There were 1017 mother-infant control pairs with both maternal and umbilical cord blood samples. The mean maternal 25(OH)D concentration was 51.2 nmol/L (± 15.9 nmol/L). The minimum maternal value was 11.4 nmol/L and the maximum was 132.0 nmol/L. The mean umbilical cord concentration was 66.5 nmol/L (± 23.6 nmol/L), with a minimum of 14.1 and a maximum of 149.0 nmol/L. Maternal 25(OH)D concentrations were on average 15.7 nmol/L lower than their offspring (± 25.9 nmol/L). Maternal concentrations ranged from 116.5 nmol/L below to 53.6 nmol/L above corresponding umbilical cord concentrations. Maternal concentrations were 86.3% that of their offspring on average (± 42.3%). The regression equation predicting neonatal 25(OH)D from

maternal 25(OH)D revealed that, on average, a 1 nmol/L increase in maternal 25(OH)D lead to a 0.34 nmol/L increase in neonatal 25(OH)D.

The Pearson correlation coefficient (r) for the association between maternal and neonatal 25(OH)D levels was 0.23 ($p < 0.01$). Adjusting for time between maternal and cord blood draw and for maternal month of blood draw increased the correlation coefficient between maternal and neonatal 25(OH)D concentrations to 0.46 ($p<0.01$). When the residuals of the unadjusted model were plotted in Figure 4.2 and visually examined there was neither clear nonlinearity nor non-constant variance.

Tables 4.20 and Table 4.21 show the unadjusted and adjusted correlations of maternal and neonatal 25(OH)D in controls stratified the sex of the baby, maternal pre-pregnancy BMI category, smoking status during pregnancy and season of delivery. The correlation between maternal 25(OH)D concentration and neonatal 25(OH)D concentration was higher with female babies than male babies (0.29 vs. 0.18; interaction $p=0.07$). This pattern persisted after adjusting for time between maternal and cord blood draw and for maternal season of blood draw ($r=0.50$ vs. 0.43; interaction $p=0.08$). There was a trend of increased correlation coefficients with increased maternal pre-pregnancy BMI category (0.19 for <25 kg/m^2, 0.23 for 25-<30 kg/m^2, 0.29 for ≥ 30kg/m^2) although maternal pre-pregnancy BMI did not significantly modify the relationship between maternal 25(OH)D and neonatal 25(OH)D (interaction $p=0.62$). These patterns remained after adjustment for covariates. The correlation between maternal and neonatal 25(OH)D was not statistically significant among mothers who smoked during pregnancy ($r=0.11$; $p=0.13$). However, smoking status during pregnancy was not shown to modify the relationship between maternal and neonatal 25(OH)D (interaction $p=0.23$). After

adjusting for covariates the correlation between maternal and neonatal 25(OH)D was statistically significant among mothers who had smoked at some point during pregnancy and their offspring ($r=0.37$; $p<0.01$). Stratifying the results by season of delivery strengthened the correlation between maternal and neonatal 25(OH)D concentrations ($r=0.38$, 0.39, 0.50 and 0.38 for late summer/ early fall, late spring/ early summer, late fall/ early winter and late winter/ early spring, respectively, vs. 0.23 for all seasons pooled). The adjusted relationship between maternal and neonatal 25(OH)D stratified by season was not adjusted for maternal or neonatal season of blood draw because this information was already contained within time between maternal and cord blood draw and season variables.

Tables 4.22 and Table 4.23 display the unadjusted and adjusted correlations of maternal and neonatal 25(OH)D by case/control status. The relationship between maternal and neonatal 25(OH)D was strongest among mother-infant pairs with a LBW infant ($r=0.30$; $p<0.01$) and weakest among pairs with a SGA infant ($r=0.21$; $p<0.01$). In the models that adjusted for time between maternal and cord blood draw and for maternal season of blood draw, the highest correlation was among pairs with a PTB infant ($r=0.53$; $p<0.01$) and lowest among pairs with a SGA infant ($r=0.42$; $p<0.01$).

Figure 4.1: Correlation between Maternal and Neonatal Vitamin D

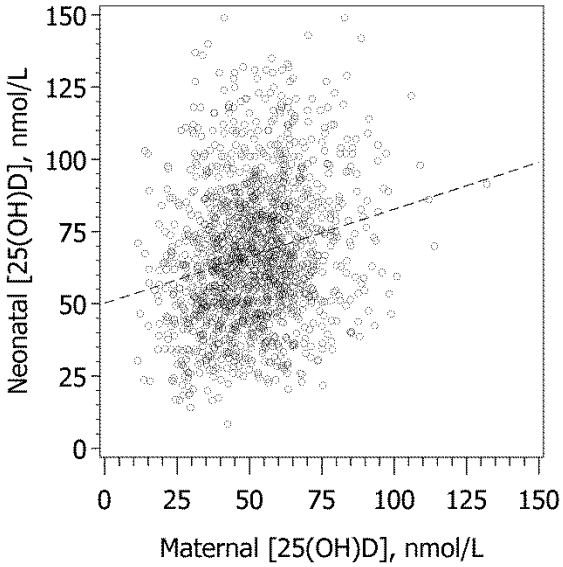

Figure 4.2: Residuals of Neonatal [25(OH)D] Predicted by Maternal [25(OH)D]

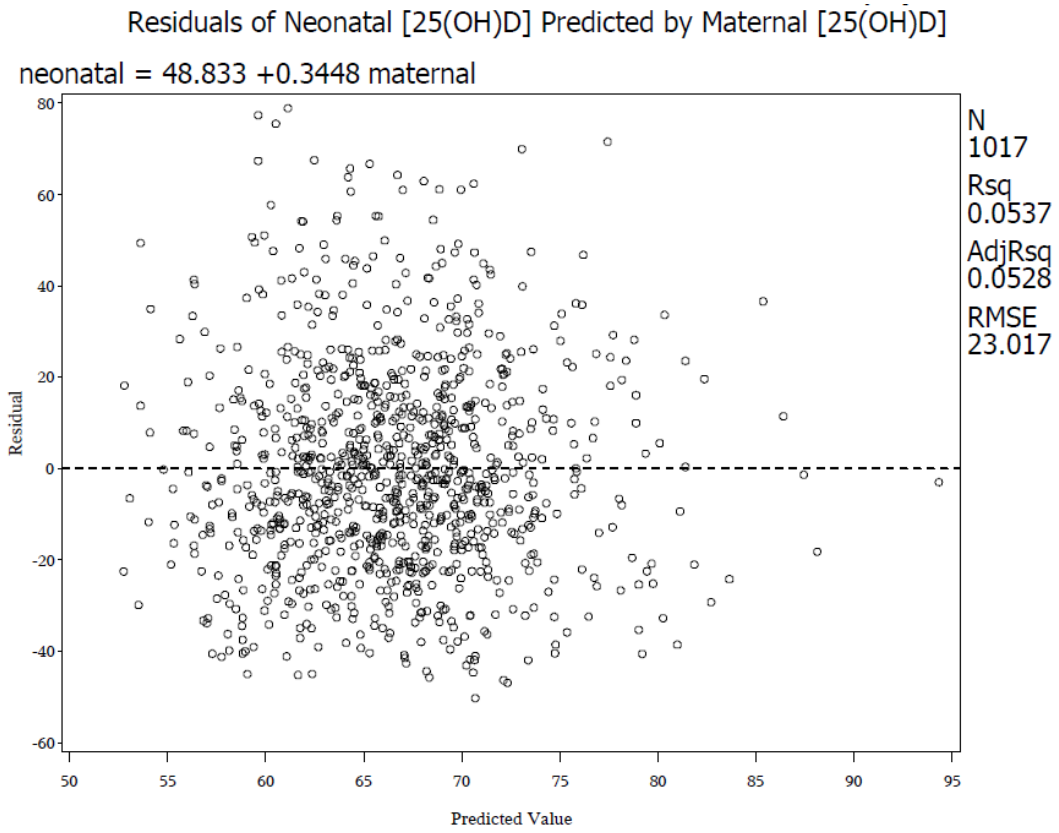

Table 4.20: Stratified Correlations of Maternal and Neonatal 25(OH)D among Controls

Group	N	Correlation	Correlation p-value	Parameter Estimates (95% CI)	Interaction p-value
Overall	1017	0.23	<0.01	0.34 (0.26, 0.43)	N/A
Male baby	523	0.18	<0.01	0.27 (0.15, 0.40)	0.07
Female baby	489	0.29	<0.01	0.44 (0.31, 0.57)	
BMI <25	633	0.19	<0.01	0.29 (0.17, 0.41)	0.62
25-<30	185	0.23	<0.01	0.32 (0.12, 0.51)	
≥30	123	0.29	<0.01	0.44 (0.18, 0.70)	
Smoking No	745	0.24	<0.01	0.36 (0.26, 0.46)	0.23
Yes	177	0.11	0.13	0.18 (-0.05, 0.42)	
Season‡ 0	246	0.38	<0.01	0.62 (0.43, 0.82)	0.71
1	318	0.39	<0.01	0.55 (0.41, 0.69)	
2	210	0.50	<0.01	0.62 (0.47, 0.77)	
3	243	0.38	<0.01	0.50 (0.35, 0.66)	

‡ Season of delivery; 0=late summer/early fall, 1=late spring/early summer, 2=late fall/early winter, and 3=late winter/early spring

Table 4.21: Stratified Correlation of Maternal and Neonatal 25(OH)D among Controls (Adjusted*)

Group	N	Correlation	Correlation p-value	Parameter Estimates (95% CI)	Interaction p-value
Overall	1017	0.46	<0.01	0.64 (0.56, 0.72)	N/A
Male baby	523	0.43	<0.01	0.59 (0.48, 0.70)	0.08
Female baby	489	0.50	<0.01	0.71 (0.60, 0.82)	
BMI <25	633	0.43	<0.01	0.61 (0.51, 0.72)	0.58
25-<30	185	0.47	<0.01	0.61 (0.43, 0.79)	
≥30	123	0.51	<0.01	0.72 (0.48, 0.97)	
Smoking No	745	0.47	<0.01	0.64 (0.56, 0.73)	0.25
Yes	177	0.37	<0.01	0.55 (0.33, 0.76)	
Season‡ 0	246	0.38	<0.01	0.62 (0.43, 0.82)	0.69
1	318	0.40	<0.01	0.57 (0.43, 0.72)	
2	210	0.50	<0.01	0.62 (0.48, 0.77)	
3	243	0.39	<0.01	0.51 (0.36, 0.67)	

*Adjusted for time between maternal and cord blood draw and for maternal month of blood draw

‡ Season of delivery, not adjusted for maternal season of blood draw or neonatal season of blood draw; 0=late summer/early fall, 1=late spring/early summer, 2=late fall/early winter, and 3=late winter/early spring

Table 4.22: Correlation of Maternal and Neonatal 25(OH)D by Case/Control Status

Group	N	Correlation	Correlation p-value	Parameter Estimates (95% CI)	Interaction p-value
Controls	1017	0.23	<0.01	0.34 (0.26, 0.43)	N/A
PTB*	221	0.26	<0.01	0.40 (0.21, 0.59)	0.60
LBW*	106	0.30	<0.01	0.46 (0.18, 0.75)	0.41
SGA*	300	0.21	<0.01	0.32 (0.15, 0.50)	0.83
Neonatal Cases	516	0.22	<0.01	0.34 (0.21, 0.46)	0.91

*Case groups are not mutually exclusive

Table 4.23: Correlation of Maternal and Neonatal 25(OH)D by Case/Control Status (Adjusted*)

Group	N	Correlation	Correlation p-value	Parameter Estimates (95% CI)	Interaction p-value
Controls	1017	0.46	<0.01	0.64 (0.56, 0.72)	N/A
PTB‡	221	0.53	<0.01	0.78 (0.60, 0.95)	0.32
LBW‡	106	0.46	<0.01	0.71 (0.41, 1.01)	0.94
SGA‡	300	0.42	<0.01	0.60 (0.45, 0.76)	0.29
Neonatal Cases	516	0.46	<0.01	0.66 (0.55, 0.77)	0.71

*Adjusted for time between maternal and cord blood draw and for maternal month of blood draw

‡Case groups are not mutually exclusive

CHAPTER FIVE: DISCUSSION

5.1 Overview of Vitamin D Status

In this nested case-control study of Quebecois newborn singleton offspring, 25(OH)D concentrations among cases and controls were within the realm of the general Canadian population, where 67% of Canadian males and 62.2% of Canadian females have 25(OH)D concentrations below 75 nmol/L (14). There were 59.5% of PTB cases, 53.8% of LBW cases, 65.1% of SGA cases and 67.5% of controls whose 25(OH)D concentrations were below this recommended value. Mean 25(OH)D levels were higher in PTB and LBW cases than in controls (69.8 ± 25.4 and 72.42 ± 25.9 vs. 66.5 ± 23.7; p=0.06 and p=0.01). Mean levels were similar between SGA cases and controls (67.2 ± 25.8 vs. 66.5 ± 23.7; p=0.66). When all of the neonatal cases were amalgamated their 25(OH)D concentrations were comparable to those of the cases. There were 63.1% of cases and 67.5% of controls with 25(OH)D concentrations <75 nmol/L (p=0.17). The mean 25(OH)D concentration among cases was 68.1 ± 25.5 nmol/L, while the mean 25(OH)D concentration among controls was 66.5 ± 23.7 nmol/L (p=0.24). The 25(OH)D concentrations of this cohort indicate that this study sample may well be representative of the Canadian population. It was somewhat surprising that mean 25(OH)D levels were higher in PTB and LBW cases than in controls and similar between SGA cases and controls. These findings were contrary to expectations.

5.2 Preterm Birth

We observed umbilical cord 25(OH)D concentrations <75 nmol/L to be protective against the risk of PTB. In the multiple imputation analysis, there appeared to be a non-

linear relationship between 25(OH)D concentration and risk of PTB across four categories of 25(OH)D (<37.5, 37.5-<50, 50-<75, ≥75 nmol/L). Concentrations that were <37.5 and 37.5-<50 nmol/L did not influence the odds of PTB compared to levels ≥75 nmol/L. However, a protective effect of 25(OH)D concentrations 50-<75 nmol/L and <75 nmol/L compared to concentrations ≥75 nmol/L was found. When categories of 25(OH)D concentrations were dichotomized at each of the four cutpoints, only levels <75 vs. ≥75 nmol/L had a statistically significant relationship. Levels <75 nmol/L suggested a protective effect on the risk of PTB compared to levels ≥75 nmol/L.

A prospective cohort conducted in Karachi, Pakistan by Hossain et al. using cord blood at delivery found that higher vitamin D status was associated with shorter gestational periods (5). This finding was similar to our multiple imputation analysis results that suggested a protective effect of 25(OH)D levels 50-<75 and <75 nmol/L compared to ≥75 nmol/L on risk of PTB. Using cord blood as the exposure variable was an important commonality of this study by Hossain et al. and the current one.

Several studies have shown that both maternal and neonatal 25(OH)D concentrations <50 nmol/L do not increase the risk of PTB compared to higher levels (19,20,22,23). The nested case-control study conducted at thirteen American centres by Thorp et al. (22) found no association between 25(OH)D levels <50 nmol/L and risk of recurrent preterm delivery. The nested case-control study conducted by Baker et al. (23) in North Carolina also found no association between 25(OH)D levels <50 nmol/L or 37.5-<50 nmol/L relative to ≥75 nmol/L. Our findings are in keeping with these previous ones - none of the ORs examining the risk of PTB based on 25(OH)D levels <50 vs. ≥50 nmol/L reached statistical significance in our study. In light of our finding that levels <75 nmol/L were

protective of PTB compared to levels ≥75 nmol/L, it is possible that <50 nmol/L represents a 25(OH)D concentration that is too low to provide optimal health benefits. Perhaps the relationship between 25(OH)D and risk of PTB is in fact non-linear. It is worth noting that the studies by Baker et al. and Thorp et al. controlled for season of measurement, which may have attenuated the relationship between 25(OH)D levels and risk of PTB due to the strong relationship between season and vitamin D status (22,23). However it is unlikely that this one factor explains the results in these studies, especially since our study did not adjust for season but still agreed with previous studies.

Two RCTs found no significant difference in gestational age among different supplementation groups despite higher percentages of 25(OH)D ≥50 nmol/L in cord blood with higher maternal supplementation level (19,20). The study by Hollis et al. (19) had three trial groups who were given 400 IU, 2,000 IU or 4,000 IU of vitamin D supplementation each day from entry into the study until delivery. The study by Yu et al. (20) had a control group, a daily dose group (800 IU of ergocalciferol) and a single group (200,000 IU of calciferol). The percentage of participants with 25(OH)D levels <50 nmol/L decreased with higher supplementation doses in both of these studies (19,20). Despite differences in prevalence of 25(OH)D <50 nmol/L among the different arms of each trial, these studies found no significant difference in gestational age at birth among the different treatment groups (19,20).

The results from a prospective cohort study conducted by Perez-Ferre et al. (21) in Madrid, Spain are in contrast to those of our study and those of several others (19,20,22,23). Perez-Ferre et al. found increased odds (OR 3.31, 95% CI 1.52-7.19; $p<0.002$) of PTB among infants whose mothers' 25(OH)D concentrations had been <50

nmol/L compared to those whose mothers' 25(OH)D concentrations had been ≥50 nmol/L (21). This general relationship between 25(OH)D concentrations and risk of PTB was consistent across all five models used in this Spanish study (crude, adjusted for un-modifiable factors, adjusted for modifiable factors, adjusted for modifiable factors including BMI as a categorical variable and adjusted for variables that differed between the groups below and above 50 nmol/L in the univariate model). The major differences between the Spanish study and ours are its use of maternal blood as opposed to our use of neonatal blood and the timing of the blood draw (at 24-28 weeks gestation vs. at birth). Two other studies that used maternal blood taken during pregnancy found no association between 25(OH)D levels <50 nmol/L vs. ≥50 nmol/L and odds of PTB (22,23). The populations in these three studies may have differed in a way that contributed to the dissimilar findings. For example, race has been shown to modify the relationship between 25(OH)D concentration and SGA status (30) and perhaps this is the case for the relationship between 25(OH)D concentration and risk of PTB. Although there has not been thorough investigation into possible effect modification between 25(OH)D levels and risk of PTB by race and/or ethnicity, race and ethnicity have been shown to be related to both 25(OH)D levels and to risk of PTB (40,51-55). Since our population was predominantly of Caucasian descent, stratifying results by race or ethnicity would have led to exceedingly small cell counts and would therefore have failed to provide insightful additional information.

A recent meta-analysis (109) included the aforementioned studies by Baker et al. and by Perez-Ferre et al. (21,23) among four studies examining the relationship between maternal 25(OH)D and PTB. The meta-analysis revealed a crude OR of 1.58 (95% CI

1.08-2.31) for babies of mothers with 25(OH)D levels <50 nmol/L. Therefore, it is possible that previous studies failing to reach statistical significance had sample sizes that were too low to detect this relationship. However, all four studies combined still only had 176 PTB babies, whereas our study had 222 PTB babies, and our study had contrasting results.

There are possible biological explanations for the potential relationship between 25(OH)D concentrations and risk of PTB. This relationship may be related to the possible impact vitamin D has on immunomodulation and the inflammatory response (109). It may also be connected to vitamin D's role on uterine immune cells (109). The cut-points chosen for 25(OH)D are based on health outcomes but are still somewhat arbitrary. The optimal level for immune functioning and by extension for minimizing the risk of PTB may hover around the 50 nmol/L or 75 nmol/L point, and this could explain contrasting results in the literature. Studies that only used one cutpoint may have had different results because they combined 25(OH)D concentrations with potentially heterogeneous effects on immune function and the risk of PTB across this range.

Several variables commonly associated with risk of PTB did not differ by PTB case/control status in the present study. These included pre-pregnancy BMI, smoking status during pregnancy, exposure to second-hand smoking during pregnancy, SES measures, ethnicity and emotional distress. However, both pre-pregnancy physical activity and physical activity during pregnancy were related to PTB case/ control status. Mothers of PTB cases had higher rates of inactivity before and during pregnancy as well as lower rates of exercising at least twice a week during pregnancy compared to controls. Season of birth also varied by case/ control status with cases having more births in the

late summer/ early fall and controls having higher rates of birth in the late spring/ early summer. The main results related to PTB in this study should be interpreted with moderate caution in the context of other studies since only two of the variables typically linked to risk of PTB differed by case/ control status in the present study.

5.3 Low Birthweight

There appeared to be a non-linear relationship between 25(OH)D level and risk of LBW across the four categories of 25(OH)D (<37.5, 37.5-<50, 50-75, ≥75 nmol/L) based on the multiple imputation analysis. Odds of LBW for neonates with 25(OH)D concentrations <37.5 nmol/L vs. ≥75 nmol/L were not statistically different (aOR 0.59, 95% CI 0.28-1.26). Odds of LBW were lower for infants with 25(OH)D levels 37.5-<50 nmol/L vs. ≥75 nmol/L (aOR 0.40, 95% 0.19-0.82) and 50-<75 nmol/L vs. ≥75 nmol/L (aOR 0.53, 95% CI 0.34-0.85). Both the complete case and multiple imputation analyses revealed lower odds of LBW with umbilical cord blood 25(OH)D concentrations <75 nmol/L vs. ≥75 nmol/L (complete case analysis aOR 0.54 95% CI 0.32-0.94; multiple imputation analysis aOR 0.51, 95% CI 0.34-0.78).

Hossain et al. (5) produced findings that were similar to those in the present study. This Pakistani study also used cord blood as its exposure variable and found increased 25(OH)D levels to be associated with decreased birthweight (5). The statistical analysis in the study by Hossain et al. differed from the present study in that the investigators examined the correlation between continuous 25(OH)D concentration and continuous birthweight.

Studies by Bowyer et al. (4) and Camargo et al. (26) found no statistically significant relationship between cord blood and risk of LBW when comparing the mean birthweight among neonates with 25(OH)D concentrations on each side of the 25 nmol/L cut-point. The non-significant results from the current study were similar to those from these two previous studies, although the lowest 25(OH)D category for the current study was <37.5 nmol/L. LBW infants were equally as likely as infants weighing >2500 g at birth to have 25(OH)D levels <37.5 nmol/L.

Three previous studies found lower 25(OH)D levels to be associated with lower birthweight (4,24,25). Bowyer et al. (4) and Gernand et al. (24) both used maternal serums as opposed to cord blood. Song et al. used neonatal serums (25). All three of these studies compared mean 25(OH)D levels between groups above and below specific birthweight cut-off points. The use of mean 25(OH)D levels may have impacted the difference in results compared to the current study as outliers could have had a major impact on the values in these other studies.

Only two of the variables commonly associated with risk of LBW were statistically different between LBW cases and controls. Mothers of LBW infants were more likely to smoke at some point during pregnancy than other mothers (27.6% vs. 19.1%; p=0.08). Mothers of LBW infants were also more likely to exercise at least twice a week before they were pregnant than mothers of control infants and also were more likely to be completely sedentary in this time-period (78.0% vs. 73.4% and 12.1% vs. 6.9%; p=0.02). On one hand, the lack of statistically significant differences between LBW case and control groups for most factors commonly associated with risk of LBW may indicate that this study population is somehow different from other populations that

have previously been examined with respect to LBW. On the other hand, the similarities between case and control groups with respect to these potential confounders may help to isolate the impact of 25(OH)D on the risk of LBW with minimal confounding by these factors.

5.4 Small for Gestational Age

None of the results from the complete case or multiple imputation analysis revealed statistically significant results. Other studies have found similar non-significant results in the association between 25(OH)D and risk of SGA (27,87). Baker et al. (27) and Farrant et al. (87) found no difference in pregnancy outcome by vitamin D concentration. Both of these studies used maternal serum taken during the third trimester of pregnancy. The timing of this blood draw, close to parturition, could have increased the similarity in null findings to the current study. A recent meta-analysis that included six studies that examined the relationship between maternal vitamin D status and SGA found no difference in the odds of delivering a SGA infant based on 25(OH)D levels <75 nmol/L compared to levels ≥75 nmol/L (109).

Several studies, including two meta-analyses, examining the association between vitamin D and risk of SGA have observed an increased risk of SGA based on lower 25(OH)D levels (28-30,109). Leffelaar et al. (28) found an aOR of 1.8 (1.3-2.5) for babies of mothers with 25(OH)D levels <30 vs. ≥50 nmol/L. This Dutch study adjusted for maternal height, parity, maternal age, smoking, maternal pre-pregnancy BMI, education level and fetal sex. It is unlikely that inclusion of additional confounders, maternal height and parity, majorly impacted differences in findings between this study

and the current one. Maternal height was factored into the calculation of pre-pregnancy BMI in the current Canadian study and it has been demonstrated that parity does not necessarily influence birth size (29). The study by Morley et al. focused on knee-heel length at birth as the outcome variable, which may not quite capture the same thing as birthweight for gestational age. All but one of the studies that found increased odds of SGA with lower 25(OH)D levels used maternal serums (28-30,109,110). It is possible that the maternal 25(OH)D drawn earlier in pregnancy is more predictive of SGA status than neonatal 25(OH)D or maternal 25(OH)D later in pregnancy.

An American study by Bodnar et al. (82) found a U-shaped relationship between maternal 25(OH)D taken at <22 weeks gestation and risk of SGA among white mothers. No association between maternal 25(OH)D concentration and risk of SGA was found for black mothers (82). Using 25(OH)D concentrations 37.5-75 nmol/L as the referent group, this study found higher odds of SGA among white women with 25(OH)D concentrations <37.5 nmol/L and >75 nmol/L (aOR 7.5, 95% 1.8-31.9; aOR 2.1, 95% CI 1.2-3.8, respectively). These findings were similar to the trend observed in our study; however the results were more pronounced in the study by Bodnar et al. (82). This study controlled for many of the same covariates as our study, which likely played an important role in the similarity in findings.

The findings from Burris et al. (31) are particularly relevant to the current study because the investigators used cord blood as an exposure variable. The authors from this study reported an OR of 4.64 (1.61-13.36) for SGA among neonates with 25(OH)D concentrations <25 nmol/L vs. ≥25 nmol/L (31).

The relationship between 25(OH)D and risk of SGA status is most likely connected to vitamin D's role in both bone development and gene regulation related to fetal growth (109). The mixed evidence concerning the optimal level of 25(OH)D and fetal growth emphasizes the complexity of this relationship.

Many of the variables commonly associated with risk of SGA were different between cases and controls. Mothers of SGA infants were more likely to be of normal weight (<25 kg/m^2), to smoke during pregnancy, to be exposed to second-hand smoke during pregnancy, to exercise two or more times a week pre-pregnancy, and also to never exercise pre-pregnancy, and to be common-law with their partner as opposed to married. These differences were taken into account in the adjusted models. These differences suggest that the current study population resembles those previously included in the examination of risk factors for SGA.

5.5 Adverse Neonatal Outcomes Overall

The multiple imputation analysis revealed somewhat of a U-shaped relationship between 25(OH)D concentration and risk of PTB, LBW and/or SGA. Infants with 25(OH)D concentrations <37.5 and 37.5-<50 nmol/L had similar odds of having an adverse neonatal outcome as infants with 25(OH)D concentrations ≥75 nmol/L (aOR 1.01, 95% CI 0.69-1.47 and aOR 0.72, 95% CI 0.51-1.02, respectively). However, infants with 25(OH)D concentrations 50-<75 nmol/L had lower odds of experiencing at least one of the adverse neonatal outcome compared to infants with 25(OH)D concentrations ≥75 nmol/L (aOR 0.77, 95% CI 0.60-0.99). Among infants whose mothers' pre-pregnancy BMIs were ≥30 kg/m^2, neonates with 25(OH)D concentrations 50-<75 nmol/L had lower

odds of PTB, LBW and/or SGA (OR 0.42, 95% CI 0.18-0.97). Since SGA infants accounted for approximately half of the cases and these cases had 25(OH)D levels that were overall comparable to controls, it is possible that combining PTB, LBW and SGA infants masked the differences between PTB and LBW case groups and controls.

Merging PTB, LBW and SGA into a single group entailed an assumption: that these three outcomes were etiologically similar with respect to vitamin D. Vitamin D's role in immune functioning (109) may serve as common pathway for 25(OH)D to influence the risk of each of the adverse neonatal outcomes included in this study. In the case of PTB 25(OH)D may impact the immune system through its potential effects on the inflammatory response (109). For LBW and SGA 25(OH)D's impact on the immune system may act more through the immune system's role in fetal development (109).

Combining adverse neonatal outcomes is helpful for capturing the overall association between 25(OH)D and perinatal health. When combining three adverse neonatal outcomes reveals almost no association between 25(OH)D concentration and risk of poor outcomes, this puts into question the need for a public health intervention. A study conducted in the U.K. also examined the relationship between 25(OH)D and PTB, LBW and SGA combined (20). This was an RCT that recruited mothers at 27 weeks gestation. Participants were randomly assigned to a stat dose of 200,000 IU of vitamin D, a daily dose of 800 IU of vitamin D until delivery, or a no treatment group (20). Neonates of mothers in the control group had the highest percentage of infants with 25(OH)D concentrations <50 nmol/L and neonates of mothers in the daily dose group had the lowest percentage of infants with 25(OH)D concentrations below this level (20). This British study found no significant difference between the trial arms (and by extension

25(OH)D concentration) and poor neonatal outcomes (20). The use of cord bloods is useful in comparing the results from this study to ours. Nevertheless the different study design (RCT vs. nested case-control) with the same overall null finding helps to corroborate the lack of association observed between 25(OH)D and risk of PTB, LBW and/or SGA.

Several variables commonly associated with the risk of PTB, LBW and/or SGA varied between case and control groups in a manner consistent with the literature. Mothers of cases were more likely to smoke during pregnancy than controls (26.0% vs. 19.1%; $p<0.01$) and to be exposed to second-hand smoke (40.1% vs. 35.5%; $p<0.09$). PTB, LBW and/or SGA infants were more likely to have mothers who exercised at least twice a week or not at all pre-pregnancy compared to control infants ($p=0.01$). Cases were also less likely to have mothers who were married, and more likely to have mothers who were common law with their partners or who were single or separated/divorced ($p=0.02$). This congruency with the literature on smoking, exercise and marital status and risk of adverse neonatal outcomes (42,49,101) suggests that the Quebec population included in the current study resembles other populations that have been studied with regards to important risk factors for adverse neonatal outcomes. This strengthens our confidence in the findings because it suggests that selection bias did not strongly affect the results.

5.6 Secondary Objective

Maternal and neonatal 25(OH)D were weakly associated, with a Pearson correlation coefficient of 0.23 ($p<0.01$) and an adjusted Pearson correlation coefficient of 0.46

($p<0.01$). Maternal 25(OH)D levels were an average of 15.7 nmol/L (± 25.9 nmol/L) lower than corresponding neonatal 25(OH)D levels and 86.3% (± 42.3%) of these corresponding levels. By stratifying correlations of maternal and neonatal 25(OH)D in controls by sex of the baby, maternal pre-pregnancy BMI category, smoking status during pregnancy and season of delivery, this study enhances the understanding of how modifiable (BMI and smoking) and non-modifiable (sex of baby and season of delivery) factors impact the relationship between maternal and neonatal 25(OH)D levels.

Most other studies examining the association between maternal and neonatal 25(OH)D pairs found positive correlations between 0.68 and 0.79 (4,5,32,35). These correlations are all higher than even the adjusted correlation for the current study. All but one of these previously conducted studies used maternal blood samples that were drawn just before or just after delivery (5,32,35). This serves as a logical explanation for the higher correlation compared to the current study. Although the study by Bowyer et al. (4) did not use maternal blood drawn at delivery, it was drawn closer to the time of delivery than the maternal blood used in the current study. Maternal blood was taken between 30 and 32 weeks in this Australian study (4). This may help to explain the stronger correlation. The Australian study did not control for confounding variables when examining the relationship between maternal and neonatal 25(OH)D, so the difference cannot be explained by adjustment.

The study by Dror et al. (35) found that fetal levels drawn at birth were on average of 61% ± 18% of corresponding maternal levels that were drawn upon admission to the labour and delivery unit. This finding contrasts the observation in the current study that fetal levels were higher than corresponding maternal levels. The differences in time

of blood draw could have influenced these opposing findings. The increased maternal metabolism of vitamin D during pregnancy, partially due to fetal demands (4,9), could vary across the course of pregnancy. If fetal demands for vitamin D are higher early to mid-pregnancy then maternal levels could decrease at this point to a greater extent than later in pregnancy. This would be done in order to meet the requirements of the developing fetus.

5.7 Strengths and Limitations

The major strengths of this study stem from the focus on cord blood, the additional examination of maternal serums, the demographics of the population being examined, and the use of samples and questionnaires from a previously conducted study. Using cord blood allowed for a more direct measure of the vitamin D that the fetus received in utero; it potentially provided a better representation of the biologically effective dose. The inclusion of the secondary objective, examining the relationship between maternal vitamin D in the first trimester and vitamin D in cord blood at birth provides insightful information for previous studies that have looked at *maternal* vitamin D and neonatal outcomes. With maternal serum samples restricted to the first trimester, the timeframe for the exposure for our second objective was narrow enough to identify this relationship at a fairly specific time during pregnancy. The mostly Caucasian population examined belongs to a demographic group that is often overlooked in vitamin D studies because other ethnic groups are at higher risk of low vitamin D status and typically have lower vitamin D concentrations (38). Furthermore, it is possible that vitamin D is in fact more crucial in this population to fetal development than in other populations (30). The nested design of this study increased the cost-efficiency of this

study. We had the advantage of having prospective data collection and having this data collection already complete, which contributed to the large sample size. Samples for this study were previously collected over a five year time period. We were able to harness the data contained in this CIHR-funded cohort study of pregnant women recruited before 20 weeks gestation in Quebec City, Quebec.

The major limitation of this study is related to selection bias. A greater proportion of cases were missing cord blood samples than controls (39.8% vs. 27.5%). It is most likely that more cord blood samples were missing among especially unhealthy babies because the birth team would have prioritized any urgent procedures over the collection of cord blood for a research study. Depending on whether the risk associated with vitamin D status shows a gradation with neonatal outcomes of increasing severity, this could have attenuated differences between cases and controls.

There was the potential for the misclassification of participants' 25(OH)D concentrations since the assays are imperfect by nature and strict cut-offs were used. However, this misclassification would not be differential between cases and controls and would therefore not be a source of information bias. Despite attempts to address confounding, there could still have been residual confounding in this study. There were differences in 'missingness' among cases and controls with respect to marital status, BMI, smoking, physical activity, SES measures and ethnicity. Multiple imputation of missing variables helped to fill in missing data as accurately as possible, but it did not fully compensate for missing values. Multiple imputation carried with it the assumption that after accounting for the factors used to fill in the missing values, the missing values were missing at random.

It is important to note the possibility of reverse causality in the relationship between vitamin D status and adverse neonatal outcomes. For example, gestational length may determine vitamin D status as opposed to vitamin D status influencing gestational length. Moreover, despite the relatively long half-life of 25(OH)D, the course of PTB, LBW and SGA may be heavily influenced earlier in the gestational period than the timing of our main exposure measure was able to capture.

5.8 External Validity

This study included mothers from Quebec City and their infants. The results of this study should only be applied to predominantly Caucasian populations due to the largely homogeneous make-up of the study population and since vitamin D has been shown to have different impact on the risk of adverse neonatal outcomes based on race (30).

5.9 Future Research

The clinical implications of this study must be considered in light of other studies that look at other important perinatal outcomes. Although this study showed lower odds of adverse neonatal outcomes among neonates with 25(OH)D concentrations <75 nmol/L compared to levels ≥75 nmol/L this does not allow for specific recommendations on vitamin D supplementation or monitoring during pregnancy. Further research, including RCTs that include vitamin D supplementation, is required in order to establish these recommendations.

Further research in this field is warranted in order to address some of the major limitations of the current study and to enhance our knowledge of the relationship between

vitamin D and neonatal outcomes as well as overall health. A prospective study would be very useful if it could capture cord blood samples from all babies delivered. This would help to address the potential for selection bias that occurred in the current study, as demonstrated by the disproportionate amount of missing cord bloods among cases compared to controls. RCTs would be useful to establish optimal supplementation levels and timing. Moreover, studies with more ethnically diverse populations would be helpful in extending the reach of applicability of studies in this area.

In order to establish the critical point of vitamin D exposure it would be ideal to obtain fetal blood samples at different points of pregnancy. However, it is this author's belief that the risk of 1% fetal loss with current blood sampling techniques (111) outweighs the benefit that knowledge of the critical point of vitamin D exposure would provide. In order to safely gain an understanding of the impact of fetal demands over the entire course of pregnancy, it would be useful to create a maternal vitamin D profile from conception to delivery. In order to examine this profile, blood samples could be taken at least once a month throughout pregnancy.

'Bench research' would be useful in generating a better understanding of the mechanisms involved in the relationship between vitamin D and risk of PTB, LBW and/or SGA. How exactly does 25(OH)D impact immune and inflammatory responses to influence rates of PTB? What specific genes are involved? How can these be targeted to lower the risk of PTB? How much of the relationship between vitamin D and fetal growth is based on bone growth? In what other ways does vitamin D impact fetal growth? These would be valuable questions worth answering to hone in on the exact relationship between vitamin D and risk of adverse neonatal outcomes.

5.10 Dissemination

Preliminary findings were presented at the Canadian Society for Epidemiology and Biostatistics student conference in St. John's, Newfoundland, in June 2013 under the theme of Reproductive, Perinatal and Child Health. Insights gathered from this research will be written up in one or two papers that will be submitted to peer-reviewed journals. Through these platforms for dissemination we aim to fulfill the overarching goal of informing research with regards to vitamin D intake during pregnancy which may help ensure adequate levels for the fetus.

3.11 Conclusions

This study set out to determine whether vitamin D status in cord blood was associated with PTB, LBW and SGA, and also to establish the relationship between maternal 25(OH)D concentrations in the first trimester of pregnancy and neonatal 25(OH)D concentrations at birth, in a birth cohort from Quebec. Compared to 25(OH)D concentrations ≥75 nmol/L, 25(OH)D concentrations 50-<75 nmol/L were associated with lower odds of PTB and a composite of PTB, LBW and/or SGA, while 25(OH)D concentrations 37.5-<50 nmol/L and 50-<75 nmol/L were associated with lower odds of LBW, and 25(OH)D concentrations <75 nmol/L were associated with lower odds of PTB and LBW. Maternal and neonatal 25(OH)D concentrations were correlated ($r=0.23$, $p<0.01$; adjusted $r=0.46$). Maternal 25(OH)D concentrations were on average 86.3% (± 42.3%) of corresponding neonatal 25(OH)D concentrations.

The results of this thesis suggest that there may be a slightly protective effect of 25(OH)D concentrations 37.5-<75 nmol/L compared to those concentrations ≥75 nmol/L.

Even so, prenatal vitamin D recommendations require an examination of the literature at large rather than just one study. Given the significant impact of adverse neonatal outcomes in the short-term and throughout the life-course, further investigation of the relationship between maternal as well as neonatal vitamin D and risk of adverse neonatal outcomes is required. It is also important to address common and pervasive risk factors for these adverse outcomes from both research and policy standpoints.

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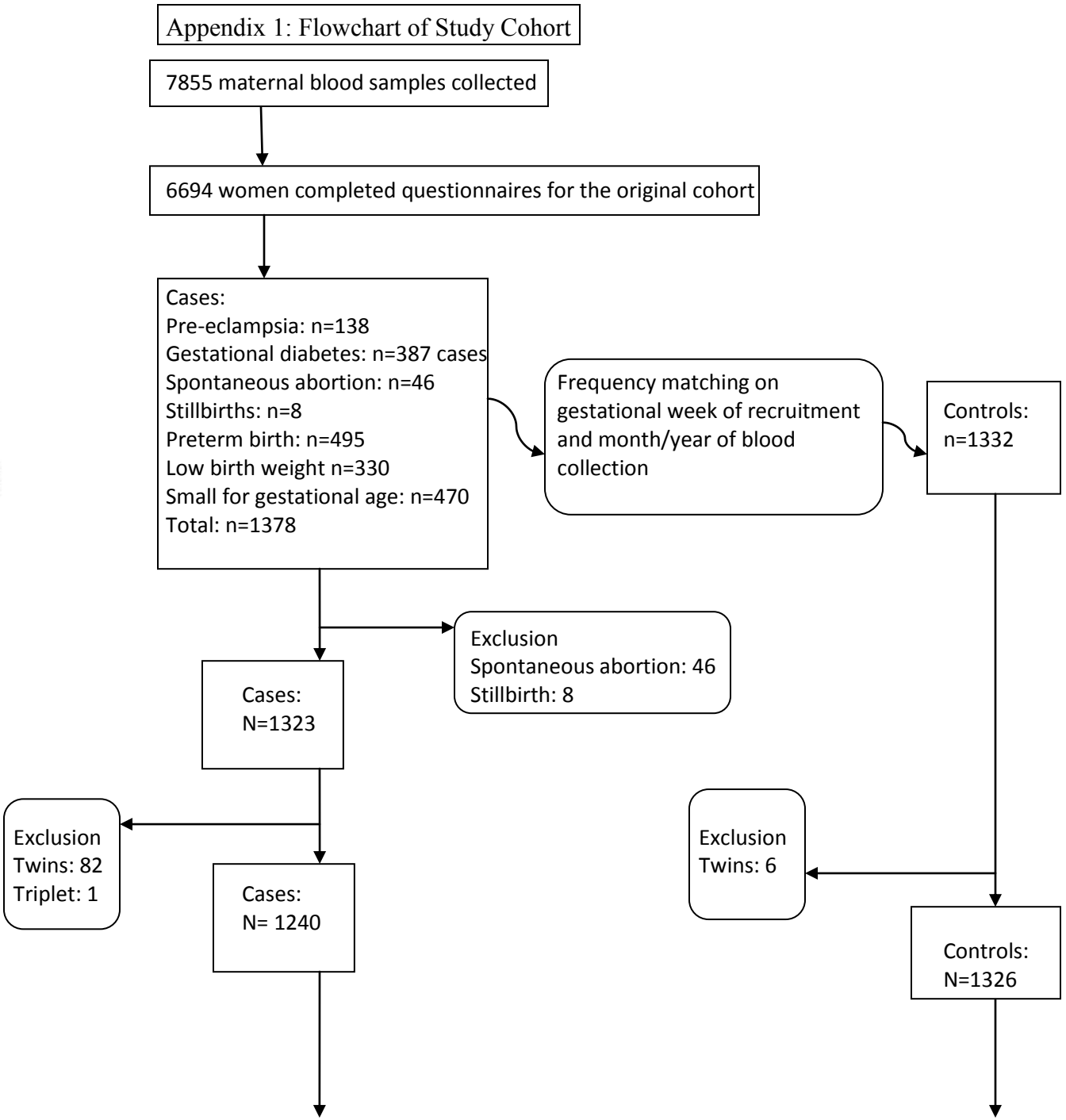
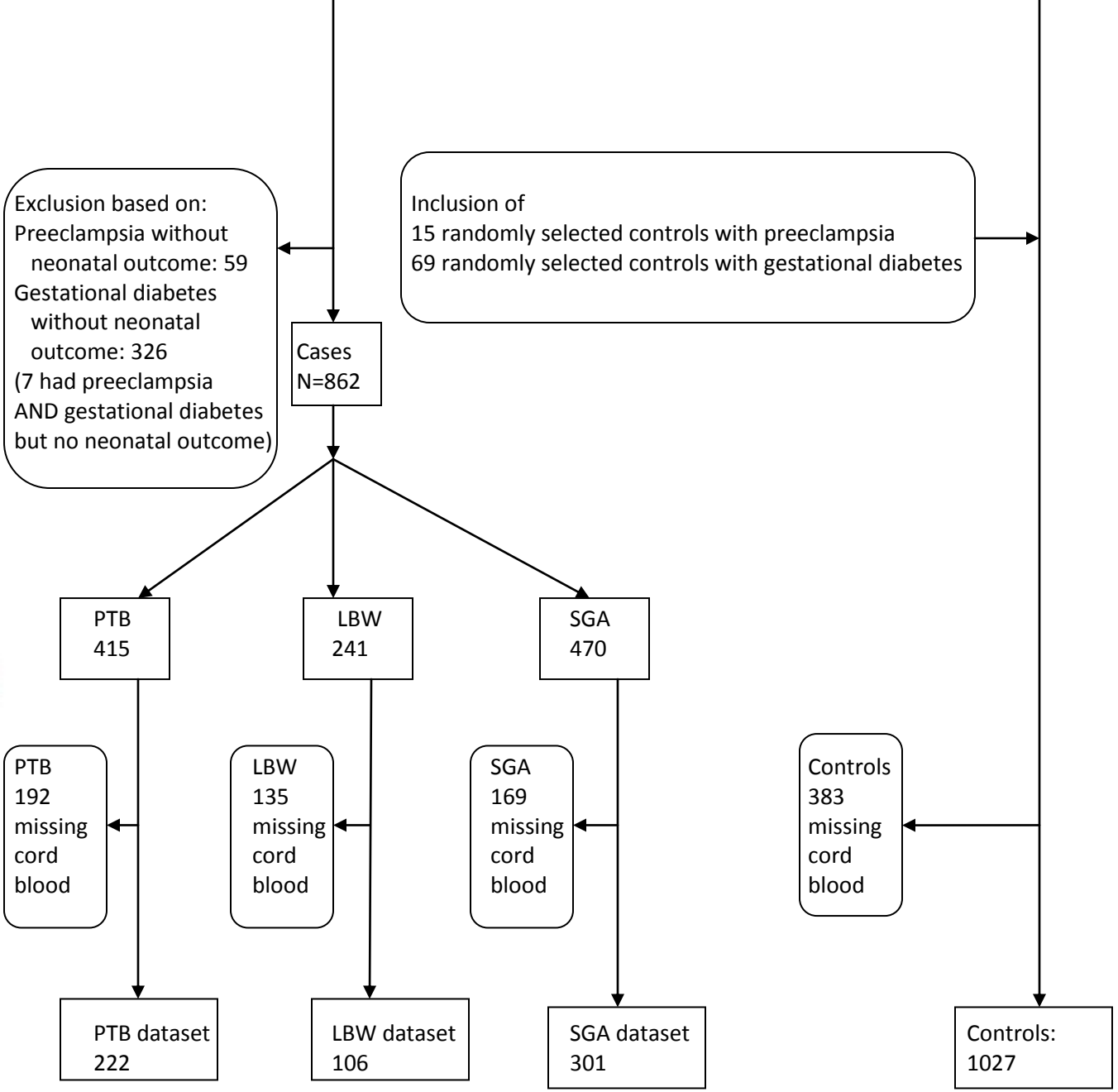
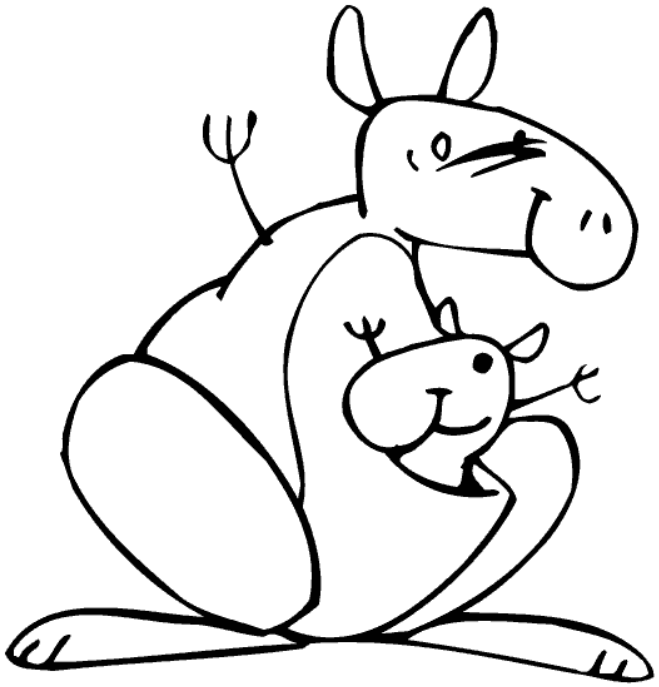
Appendix 1: Flowchart of Study Cohort




Exclusion based on:
Preeclampsia without neonatal outcome: 59
Gestational diabetes without neonatal outcome: 326
(7 had preeclampsia AND gestational diabetes but no neonatal outcome)
Inclusion of
15 randomly selected controls with preeclampsia
69 randomly selected controls with gestational diabetes
Cases
N=862
PTB
415
LBW
241
SGA
470
PTB
192
missing
cord
blood
LBW
135
missing
cord
blood
SGA
169
missing
cord
blood
Controls
383
missing
cord
blood
PTB dataset
222
LBW dataset
106
SGA dataset
301
Controls:
1027

Appendix 2 Quebec City Questionnaire

Human biological specimen bank for the study of complications of pregnancy, maternal-fetal exchanges and their subsequent consequences



General questionnaire

To be completed by the patient

If you do not understand a question or if you need any explanations, do not hesitate to ask the person who gave you the questionnaire for help.
Thank you for your cooperation.

DATE OF MEETING: | D | D | / | M | M | / | Y | Y | Y | Y |

A. First, we would like to know more about you…

Origins

Date of birth

1. What is **your** date of birth? | D | D | / | M | M | / | Y | Y | Y | Y |

Place of birth

2. What is your place of birth?

City 2.1:_____ Province 2.2:_____ Country 2.3:_____

Ethnicity

3. What is your parents' ethnicity?

	Mother 3.1	**Father** 3.2
Caucasian (white)	☐	☐
First Nations	☐	☐
African-Canadian	☐	☐
Latin-Canadian	☐	☐
Asian	☐	☐
I don't know	☐	☐
Other (specify)	_____	_____

Languages spoken

4. What is your mother tongue? _____

5. What language(s) do you speak at home? _____

Anthropometric data

6. Weight before pregnancy

6.1 How much did you weigh when you were 18 years old?

☐☐☐ **pounds or** 6.1 ☐☐☐.☐ **kilos**

6.2 How much did you weigh before this pregnancy?

☐☐☐ **pounds or** 6.2 ☐☐☐.☐ **kilos**

Height

7. What is your height?

7.1 ☐ **feet** 7.2 ☐☐ **inches or** 7.3 ☐☐☐ **cm**

Socioeconomic data

Marital status

8. What is your marital status?

- Single ☐
- Married ☐
- Common-law partner (living with someone) ☐
- Separated/Divorced ☐
- Widowed ☐

Education

9. What is your highest educational qualification?

- None (did not finish high school) ☐
- High school (including vocational studies) ☐
- College (CEGEP) ☐
- University ☐

Occupation

10. At the beginning of your pregnancy…	**YES**	**NO**	
I was a part-time student	☐	☐	10.1
I was a full-time student	☐	☐	10.2
I was unemployed (and not looking for work)	☐	☐	10.3
I was unemployed but looking for work	☐	☐	10.4
I had a paid, part-time job	☐	☐	10.5
I had a paid, full-time job	☐	☐	10.6
I had more than one job	☐	☐	10.7

11. At the moment…

	YES	NO	
I am a part-time student	☐	☐	11.1
I am a full-time student	☐	☐	11.2
I am unemployed (and not looking for work)	☐	☐	11.3
I am unemployed but looking for work	☐	☐	11.4
I have a paid, part-time job	☐	☐	11.5
I have a paid, full-time job	☐	☐	11.6
I have more than one job	☐	☐	11.7
I am on preventative withdrawal from work	☐	☐	11.8
I have been reassigned	☐	☐	11.9

Change of occupation

12. Is your answer to question #10 different to your answer to question #11?

YES ☐ **NO** ☐

12.1 If yes, how long ago did the situation change?

(Round off to 1 week if several days)

☐☐ **week(s)**

Work schedule

13. If you are working at the moment, how many hours a week do you work? (If more than one job, write the total number of hours worked.)

☐☐ **hour(s)/week**

13.1 At the beginning of your pregnancy, how many hours a week did you work? (If more than one job, write the total number of hours worked.)

☐☐ **hour(s)/week**

Family income

14. What is your gross **family** income (last year)?

Less than $15,499	☐
$15,500 to $24,999	☐
$25,000 to $39,999	☐
$40,000 to $59,999	☐
$60,000 or more	☐
I don't know	☐
I refuse to answer this question	☐

B. We would also like to know about your living arrangements…

Your household

Home postal code

1. What are the first three characters of your postal code? (e.g., G8Y) ☐☐☐

Household members

2. Who do you live with?

I live:	YES	NO	
alone	☐	☐	2.1.1
with a partner (male)	☐	☐	2.1.2
with a partner (female)	☐	☐	2.1.3
with a child	☐	☐	2.1.4
with several children	☐	☐	2.1.5
with friends	☐	☐	2.1.6
with my parents	☐	☐	2.1.7

2.1 How many people do you live with (**excluding yourself**)?

☐☐ **person(s)**

Length of relationship: *Some complications of pregnancy are related to the length of the relationship before pregnancy.*

3.1 How long have you been having sexual relations with your child's father?

3.1.1 ☐☐ **years** 3.1.2 ☐☐ **months**

3.2 How long had you been trying to get pregnant?

3.2.1 ☐☐ **years** 3.2.2 ☐☐ **months**

Type of housing

4. What type of housing do you live in?

- In a single-family house ☐
- In a building with 2 or 3 apartments ☐
- In a building with 4 to 6 apartments ☐
- In a building with more than 6 apartments ☐

5. How many bedrooms are there in your home?

- One ☐
- Two ☐
- Three ☐
- More than three ☐

Job requirements

6. Does your **current** job involve **Does not apply** ☐

	YES	**NO**
… physical exertion (carrying or lifting loads of over 10 kg)	☐	☐ 6.1
… long periods of standing	☐	☐ 6.2
… long periods of sitting	☐	☐ 6.3
… working at night (between midnight and 6 in the morning)	☐	☐ 6.4

C. Lifestyle

Tobacco smoking

Current situation

1. With regards to smoking, are you:

A non-smoker	☐	*Go to question 2.*
An ex-smoker	☐	*Go to question 1.1.*
A smoker (including occasional)	☐	*Go to question 1.2.*

Ex-smoker

1.1 If you are an ex-smoker, when did you stop smoking?

During the 2nd trimester	☐
During the 1st trimester	☐
When I found out I was pregnant	☐
Less than 6 months before my pregnancy	☐
6 to 12 months before my pregnancy	☐
Over a year before my pregnancy	☐

Smoker

1.2 If you smoke, how many cigarettes do you smoke on average per day?

Less than 1 per day	☐
1 to 10	☐
11 to 25	☐
26 or more	☐

Exposure to smoke during pregnancy

2. During your pregnancy, were you exposed to the smoke of other smokers?

Yes	☐	
No	☐	*Go to question 3.*

2.1 Were you exposed to the smoke of other smokers at home? **YES** ☐ **NO** ☐

2.2 If yes, for how many hours per day (on average)? ☐☐ **hours/day**

2.3 Were you exposed to the smoke of other smokers at work? **YES** ☐ **NO** ☐

2.4 If yes, for how many hours per day (on average)? ☐☐ **hours/day**

2.5 Were you exposed to the smoke of other smokers during your leisure activities? (including rarely) **YES** ☐ **NO** ☐

2.6 If yes, for how many hours per week (on average)? ☐☐ **hours/week**

Physical exercise

BEFORE your pregnancy

3. **In the 3 months before your pregnancy,** how many times did you do physical activity for 20 to 30 minutes **in your free time?**

Never ☐
Around once/month ☐
Around 2-3 times/month ☐
Around once/week ☐
Around 2-3 times/week ☐
4 times or more/week ☐

3.1 If yes, what activity(ies)? _____

DURING your pregnancy

4. **In the last 3 months,** how many times have you done physical activity for 20 to 30 minutes **in your free time?**

Never ☐
Around once/month ☐
Around 2-3 times/month ☐
Around once/week ☐
Around 2-3 times/week ☐
4 times or more/week ☐

4.1 If yes, what activity(ies)? _____

Nutrition

5. How much of the following do you eat/drink on average?

	BEFORE 5.x.1/2 THIS PREGNANCY	**DURING** 5.x.3/4 THIS PREGNANCY
Example: Cup(s) of coffee	__10__ number ☐ per day ☑ per week	___1___ number ☑ per day ☐ per week
Cup(s) of coffee 5.1	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week
Cup(s) of tea 5.2	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week
Glass(es) of water 5.3	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week
Glass(es) of carbonated drink 5.4	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week
Dairy products[1] 5.5	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week
Alcoholic drink(s)[2] 5.6	_____ number ☐ per day ☐ per week	_____ number ☐ per day ☐ per week

[1] One serving equals 1 glass of milk, 1 yogurt or 1 slice of cheese.

[2] One drink equals 12 ounces of beer, 5 ounces of wine, 1 ounce of hard liquor.

Decaffeinated coffee

6. Is the coffee you usually drink decaffeinated? **YES** ☐ **NO** ☐ **Does not apply** ☐

Drug use

Use

7. Have you ever used illegal drugs ? **YES** ☐ **NO** ☐

If not, go to question 8.

Type of drug

7.1 If yes, specify the type of drug(s) (at least one)

_____

Period of use

7.2 If yes, during which period? (Check all that apply.)

	YES	NO
Over three months before your pregnancy	☐	☐ 7.2.1
In the three months before your pregnancy	☐	☐ 7.2.2
In the first three months of your pregnancy	☐	☐ 7.2.3
Between the 4th and 6th months of your pregnancy	☐	☐ 7.2.4

7.3 If you are using at the moment, what is your frequency of use?

- Every day ☐
- At least once/week ☐
- 1 to 3 times/month ☐
- Less than once/month ☐

Dietary supplements, vitamins and natural products

8. In the last month, have you taken any supplements, vitamins or natural products?

YES ☐ **NO** ☐

	8.x.1 **Name of the supplement/vitamin/ natural product**	8.x.2 **Dose (mg or mL)**	8.x.3 **Quantity (number of times/day)**
8.1			
8.2			
8.3			
8.4			

D. Your gynecological and obstetrical history

Menstrual cycle

First menstrual period

1. How old were you when you had your first menstrual period? ☐☐ **years old**

Regularity

2. Are your menstrual periods usually regular (give or take a few days)?

YES ☐ **NO** ☐

Pregnancies

First child

3. What age were you (or will you be) when you had (or have) your first child?

☐☐ **years old**

Number of pregnancies

4. How many times have you been pregnant (**including** this one)? ☐☐ **time(s)**

Number of children

5. How many children do you have (**excluding** this one)?

☐☐ **child(ren)**

Number of live-born children

6. How many premature (less than 37 weeks) live-born children have you had?

☐☐ **child(ren)**

Number of elective abortions

7. How many voluntary interruptions of pregnancy (elective abortions) have you had?

☐☐ **abortion(s)**

Number of miscarriages

8. How many miscarriages have you had?

☐☐ **miscarriage(s)**

Breastfeeding

9. Have you ever breastfed? **YES** ☐ **NO** ☐

9.1 If yes, for how long (**in total, for all your children**)?

☐☐ **months**

Contraception

Oral contraceptives or contraceptive patches

10. Have you ever taken oral contraceptives or applied patches to your skin?

YES ☐ **NO** ☐

10.1 If yes, at what age did you start?

☐☐ **years old**

10.2 If yes, how long (in total) did you use them for?

☐☐ **years**

Contraceptive injections or implants

11. Have you ever used contraceptive injections or implants inserted under the skin?

YES ☐ **NO** ☐

11.1 If yes, at what age did you start?

☐☐ **years old**

11.2 If yes, how long (in total) did you use them for?

☐☐ **years**

Planning

12. Was this pregnancy planned? **YES** ☐ **NO** ☐

Assisted reproduction technologies

13. Did you use assisted reproduction technologies to become pregnant?

YES ☐ **NO** ☐

13.1 If yes, which technology did you use?

Artificial insemination ☐

In vitro fertilization ☐

Ovulation-inducing agents ☐

13.2 If other, specify: _____

E. Your medical history

Have you ever suffered from…	YES	NO	If yes, during which pregnancy?
Pregnancy hypertension (high blood pressure)			
1. Pregnancy induced hypertension?	☐	☐	_____ 1.1
Preeclampsia			
2. Preeclampsia?	☐	☐	_____ 2.1
Eclampsia			
3. Eclampsia?	☐	☐	_____ 3.1
HELLP syndrome			
4. HELLP syndrome?	☐	☐	_____ 4.1
Pregnancy diabetes			
5. Gestational diabetes (pregnancy diabetes)?	☐	☐	_____ 5.1

	YES	NO	If yes, since what age?
Diabetes			
6. Diabetes (outside of pregnancy)?	☐	☐	_____ years old 6.1 (Age of onset)
Chronic hypertension (high blood pressure)			
7. Chronic hypertension (not pregnancy related)?	☐	☐	_____ years old 7.1 (Age of onset)

7.2 If yes, what medication do you take for your high blood pressure?

_____

Hypercholesterolemia			
8. Hypercholesterolemia (high cholesterol)?	☐	☐	_____ years old 8.1 (Age of onset)

8.2 If yes, do you take medication for your hypercholesterolemia?

☐ ☐

8.3 If yes, what medication do you take for your hypercholesterolemia?

_____

	YES	NO	If yes, since what age?
Heart disease			
9. Heart diseases?	☐	☐	_____years old 9.1 Age of onset
Kidney disease			
10. Kidney diseases?	☐	☐	_____years old 10.1 Age of onset
Oral disease			
11. Oral diseases?	☐	☐	_____years old 11.1 Age of onset

11.2 If yes, which one(s)? (chronic gingivitis, periodontitis)

_____

Sexually transmitted diseases		
12. Sexually transmitted diseases?	☐	☐

12.1 If yes, which one(s)?

_____

F. Your family (and your child's father's family) medical history

For each of the questions below, do you know if any family member has or has had the following health problems?

- Your family refers to **your** immediate family ***(excluding relatives by marriage)***.
- **Your child's father's** family refers to his immediate family ***(excluding relatives by marriage***).
- If you or your partner were **adopted** or have an **unknown medical history** (*e.g., insemination*), please check "**I don't know**" at the appropriate places.

Hypertensive disorders of pregnancy

1. History of pregnancy hypertension in the family?

			Yes	No	I don't know
Your family					
1.1	*Your family?*		☐	☐	☐
	If yes, in:…	Grandmother	☐	☐	☐ 1.1.1
		Mother	☐	☐	☐ 1.1.2
		Aunt	☐	☐	☐ 1.1.3
		Sister	☐	☐	☐ 1.1.4
		Cousin (female)	☐	☐	☐ 1.1.5
Your child's father's family					
1.2	*Your child's father's family?*		☐	☐	☐
	If yes, in:…	Grandmother	☐	☐	☐ 1.2.1
		Mother	☐	☐	☐ 1.2.2
		Aunt	☐	☐	☐ 1.2.3
		Sister	☐	☐	☐ 1.2.4
		Cousin (female)	☐	☐	☐ 1.2.5

Preeclampsia

2. History of preeclampsia in the family?

		Yes	No	I don't know	
Your family					
2.1	*Your family?*	☐	☐	☐	
If yes, in:…	Grandmother	☐	☐	☐	2.1.1
	Mother	☐	☐	☐	2.1.2
	Aunt	☐	☐	☐	2.1.3
	Sister	☐	☐	☐	2.1.4
	Cousin (female)	☐	☐	☐	2.1.5
Your child's father's family					
2.2	*Your child's father's family?*	☐	☐	☐	
If yes, in:…	Grandmother	☐	☐	☐	2.2.1
	Mother	☐	☐	☐	2.2.2
	Aunt	☐	☐	☐	2.2.3
	Sister	☐	☐	☐	2.2.4
	Cousin (female)	☐	☐	☐	2.2.5

Pregnancy diabetes

3. History of pregnancy diabetes in the family?

		Yes	No	I don't know
Your family				
3.1 *Your family?*		☐	☐	☐
If yes, in:…	Grandmother	☐	☐	☐ 3.1.1
	Mother	☐	☐	☐ 3.1.2
	Aunt	☐	☐	☐ 3.1.3
	Sister	☐	☐	☐ 3.1.4
	Cousin (female)	☐	☐	☐ 3.1.5
Your child's father's family				
3.2 *Your child's father's family?*		☐	☐	☐
If yes, in:…	Grandmother	☐	☐	☐ 3.2.1
	Mother	☐	☐	☐ 3.2.2
	Aunt	☐	☐	☐ 3.2.3
	Sister	☐	☐	☐ 3.2.4
	Cousin (female)	☐	☐	☐ 3.2.5

Chronic hypertension

4. History of hypertension (high blood pressure) in the family?

		Yes	No	I don't know	Age at diagnosis
Your family					
4.1 *Your family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 4.1.1	
	Mother	☐	☐	☐ 4.1.2	If yes:_____years old 4.1.2.1
	Aunt	☐	☐	☐ 4.1.3	
	Sister	☐	☐	☐ 4.1.4	If yes:_____years old 4.1.4.1
	Cousin (female)	☐	☐	☐ 4.1.5	
	Grandfather	☐	☐	☐ 4.1.6	
	Father	☐	☐	☐ 4.1.7	If yes:_____years old 4.1.7.1
	Uncle	☐	☐	☐ 4.1.8	
	Brother	☐	☐	☐ 4.1.9	If yes:_____years old 4.1.9.1
	Cousin	☐	☐	☐ 4.1.10	
The child's father					
4.2 *Your child's father*?		☐	☐	☐	If yes:_____years old 4.2.1
Your child's father's family?					
4.3 *Your child's father's family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 4.3.1	
	Mother	☐	☐	☐ 4.3.2	If yes:_____years old 4.3.2.1
	Aunt	☐	☐	☐ 4.3.3	
	Sister	☐	☐	☐ 4.3.4	If yes:_____years old 4.3.4.1
	Cousin (female)	☐	☐	☐ 4.3.5	
	Grandfather	☐	☐	☐ 4.3.6	
	Father	☐	☐	☐ 4.3.7	If yes:_____years old 4.3.7.1
	Uncle	☐	☐	☐ 4.3.8	
	Brother	☐	☐	☐ 4.3.9	If yes:_____years old 4.3.9.1
	Cousin	☐	☐	☐ 4.3.10	

Diabetes

5. History of diabetes in the family?

		Yes	No	I don't know	Age at diagnosis
Your family					
5.1 *Your family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 5.1.1	
	Mother	☐	☐	☐ 5.1.2	If yes:_____years old 5.1.2.1
	Aunt	☐	☐	☐ 5.1.3	
	Sister	☐	☐	☐ 5.1.4	If yes:_____years old 5.1.4.1
	Cousin (female)	☐	☐	☐ 5.1.5	
	Grandfather	☐	☐	☐ 5.1.6	
	Father	☐	☐	☐ 5.1.7	If yes:_____years old 5.1.7.1
	Uncle	☐	☐	☐ 5.1.8	
	Brother	☐	☐	☐ 5.1.9	If yes:_____years old 5.1.9.1
	Cousin	☐	☐	☐ 5.1.10	
The child's father					
5.2 *Your child's father*?		☐	☐	☐	If yes:_____years old 5.2.1
Your child's father's family?					
5.3 *Your child's father's family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 5.3.1	
	Mother	☐	☐	☐ 5.3.2	If yes:_____years old 5.3.2.1
	Aunt	☐	☐	☐ 5.3.3	
	Sister	☐	☐	☐ 5.3.4	If yes:_____years old 5.3.4.1
	Cousin (female)	☐	☐	☐ 5.3.5	
	Grandfather	☐	☐	☐ 5.3.6	
	Father	☐	☐	☐ 5.3.7	If yes:_____years old 5.3.7.1
	Uncle	☐	☐	☐ 5.3.8	
	Brother	☐	☐	☐ 5.3.9	If yes:_____years old 5.3.9.1
	Cousin	☐	☐	☐ 5.3.10	

Hypercholesterolemia

6. History of hypercholesterolemia (high cholesterol) or hypertriglyceridemia in the family?

		Yes	No	I don't know	Age at diagnosis
Your family					
6.1 *Your family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 6.1.1	
	Mother	☐	☐	☐ 6.1.2	If yes:_____years old 6.1.2.1
	Aunt	☐	☐	☐ 6.1.3	
	Sister	☐	☐	☐ 6.1.4	If yes:_____years old 6.1.4.1
	Cousin (female)	☐	☐	☐ 6.1.5	
	Grandfather	☐	☐	☐ 6.1.6	
	Father	☐	☐	☐ 6.1.7	If yes:_____years old 6.1.7.1
	Uncle	☐	☐	☐ 6.1.8	
	Brother	☐	☐	☐ 6.1.9	If yes:_____years old 6.1.9.1
	Cousin	☐	☐	☐ 6.1.10	
The child's father					
6.2 *Your child's father*?		☐	☐	☐	If yes:_____years old 6.2.1
Your child's father's family?					
6.3 *Your child's father's family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 6.3.1	
	Mother	☐	☐	☐ 6.3.2	If yes:_____years old 6.3.2.1
	Aunt	☐	☐	☐ 6.3.3	
	Sister	☐	☐	☐ 6.3.4	If yes:_____years old 6.3.4.1
	Cousin (female)	☐	☐	☐ 6.3.5	
	Grandfather	☐	☐	☐ 6.3.6	
	Father	☐	☐	☐ 6.3.7	If yes:_____years old 6.3.7.1
	Uncle	☐	☐	☐ 6.3.8	
	Brother	☐	☐	☐ 6.3.9	If yes:_____years old 6.3.9.1
	Cousin	☐	☐	☐ 6.3.10	

Heart disease

7. History of heart disease (infarct, angina, heart attack or other) in the family?

		Yes	No	I don't know	Age at diagnosis
Your family					
7.1 *Your family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 7.1.1	
	Mother	☐	☐	☐ 7.1.2	If yes:_____years old 7.1.2.1
	Aunt	☐	☐	☐ 7.1.3	
	Sister	☐	☐	☐ 7.1.4	If yes:_____years old 7.1.4.1
	Cousin (female)	☐	☐	☐ 7.1.5	
	Grandfather	☐	☐	☐ 7.1.6	
	Father	☐	☐	☐ 7.1.7	If yes:_____years old 7.1.7.1
	Uncle	☐	☐	☐ 7.1.8	
	Brother	☐	☐	☐ 7.1.9	If yes:_____years old 7.1.9.1
	Cousin	☐	☐	☐ 7.1.10	
The child's father					
7.2 *Your child's father*?		☐	☐	☐	If yes:_____years old 7.2.1
Your child's father's family?					
7.3 *Your child's father's family?*		☐	☐	☐	
If yes, in:	Grandmother	☐	☐	☐ 7.3.1	
	Mother	☐	☐	☐ 7.3.2	If yes:_____years old 7.3.2.1
	Aunt	☐	☐	☐ 7.3.3	
	Sister	☐	☐	☐ 7.3.4	If yes:_____years old 7.3.4.1
	Cousin (female)	☐	☐	☐ 7.3.5	
	Grandfather	☐	☐	☐ 7.3.6	
	Father	☐	☐	☐ 7.3.7	If yes:_____years old 7.3.7.1
	Uncle	☐	☐	☐ 7.3.8	
	Brother	☐	☐	☐ 7.3.9	If yes:_____years old 7.3.9.1
	Cousin	☐	☐	☐ 7.3.10	

Kidney disease

8. History of kidney disease (pyelonephritis, etc.) in the family?

			Yes	No	I don't know	Age at diagnosis
Your family						
8.1	*Your family?*		☐	☐	☐	
	If yes, in	Grandmother	☐	☐	☐ 8.1.1	
		Mother	☐	☐	☐ 8.1.2	If yes:_____years old 8.1.2.1
		Aunt	☐	☐	☐ 8.1.3	
		Sister	☐	☐	☐ 8.1.4	If yes:_____years old 8.1.4.1
		Cousin (female)	☐	☐	☐ 8.1.5	
		Grandfather	☐	☐	☐ 8.1.6	
		Father	☐	☐	☐ 8.1.7	If yes:_____years old 8.1.7.1
		Uncle	☐	☐	☐ 8.1.8	
		Brother	☐	☐	☐ 8.1.9	If yes:_____years old 8.1.9.1
		Cousin	☐	☐	☐ 8.1.10	
The child's father						
8.2	*Your child's father*?		☐	☐	☐	If yes:_____years old 8.2.1
Your child's father's family?						
8.3	*Your child's father's family?*		☐	☐	☐	
	If yes, in:	Grandmother	☐	☐	☐ 8.3.1	
		Mother	☐	☐	☐ 8.3.2	If yes:_____years old 8.3.2.1
		Aunt	☐	☐	☐ 8.3.3	
		Sister	☐	☐	☐ 8.3.4	If yes:_____years old 8.3.4.1
		Cousin (female)	☐	☐	☐ 8.3.5	
		Grandfather	☐	☐	☐ 8.3.6	
		Father	☐	☐	☐ 8.3.7	If yes:_____years old 8.3.7.1
		Uncle	☐	☐	☐ 8.3.8	
		Brother	☐	☐	☐ 8.3.9	If yes:_____years old 8.3.9.1
		Cousin	☐	☐	☐ 8.3.10	

F. Your stress level

Personal condition

The way you felt last week may be different to the way you felt last year. Basing your answers on **last week**, use the scale below to indicate how often the following statements apply to you (circle your answers).

1 Never	2 Sometimes	3 Fairly often	4 Very often

1.	Did you feel hopeless when you thought about the future?	1	2	3	4
2.	Did you feel lonely?	1	2	3	4
3.	Did you have any memory lapses?	1	2	3	4
4	Did you feel fed up or "down"?	1	2	3	4
5.	Did you feel tense or under pressure?	1	2	3	4
6.	Did you lose your temper with someone or over something?	1	2	3	4
7.	Did you feel bored or uninterested in things?	1	2	3	4
8.	Did you feel frightened or worried?	1	2	3	4
9.	Did you have trouble remembering things?	1	2	3	4
10.	Did you cry easily or feel you were about to cry?	1	2	3	4
11.	Did you feel restless or nervous on the inside?	1	2	3	4
12.	Did you feel negative toward other people?	1	2	3	4
13.	Did you feel easily annoyed or irritated?	1	2	3	4
14.	Did you get angry about unimportant things?	1	2	3	4
15.	Did you feel relaxed?	1	2	3	4
16	Did you feel overwhelmed; did you feel you didn't have	1	2	3	4

	enough time?				
17	Did you experience any physical pain: back pain, headaches, neck pain, upset stomach?	1	2	3	4
18	Did you feel worried, troubled or anxious?	1	2	3	4
19	Did you feel you no longer knew where you were at, did you feel confused, did you lack focus and concentration?	1	2	3	4
20	Did you feel full of energy, in good form?	1	2	3	4
21	Did you feel you had a great weight on your shoulders?	1	2	3	4
22	Did you have trouble controlling your reactions, your emotions, your moods, your actions?	1	2	3	4
23	Did you feel stressed?	1	2	3	4

Thank you for taking the time to answer this questionnaire.

		–									

Patient identification **Code**

Appendix 3 Quebec City Chart Review

Human biological specimen bank for the study of complications of pregnancy, maternal-fetal exchanges and their subsequent consequences

PATIENT'S AND BABY'S HOSPITAL RECORDS

DATE OF DATA COLLECTION:

D	D	/	M	M	/	Y	Y	Y	Y

		–									

Patient identification **Code**

Human biological specimen bank for the study of complications of pregnancy, maternal-fetal exchanges and their subsequent consequences

1. Information about the mother

A. Maternal medical history

Date of birth

1. Mother's date of birth: D D / M M / Y Y Y Y

Anthropometric data

2. Anthropometric data

Weight

2.1 Weight before pregnancy ___ ___ ___ . ___ **kg**

Height

2.2 Height before pregnancy ___ ___ ___ **cm**

Hypertension

Chronic hypertension

3. Hypertension before pregnancy: Yes: □1 No: □2 Not documented: □3

3.1 If yes, date of diagnosis: M M / Y Y Y Y

Hypertension treatment

3.2 If yes, treated with which medication?

3.2.1 Name of medication(s) used during pregnancy:

_____

Blood pressure BEFORE pregnancy

3.3 Most recent blood pressure **BEFORE** pregnancy (mm Hg):

3.3.1 Systolic				**mm Hg**
3.3.2 Diastolic				**mm Hg**

Patient identification **Code**

Human biological specimen bank for the study of complications of pregnancy, maternal-fetal exchanges and their subsequent consequences

Diseases or disorders BEFORE pregnancy

Diseases or disorders BEFORE this pregnancy	YES	NO	Not documented
Cardiovascular diseases			
4. Cardiovascular diseases:	☐1	☐2	☐3
4.1 Specify: _____			
Kidney diseases			
5. Kidney diseases:	☐1	☐2	☐3
Neurological diseases			
6. Neurological diseases	☐1	☐2	☐3
6.1 Specify: _____			
7. CVA or cerebral hemorrhage:	☐1	☐2	☐3
Diabetes			
8. Previous diabetes (non-gestational):	☐1	☐2	☐3
8.1 Type: _____			
9. Gestational diabetes:	☐1	☐2	☐3
Thrombophilic disorders			
10. Thrombophlebitis	☐1	☐2	☐3
10.1 Location: _____			
11. Protein C deficiency:	☐1	☐2	☐3
12. Activated protein C resistance:	☐1	☐2	☐3
13. Protein S deficiency:	☐1	☐2	☐3
14. Antiphospholipid antibodies:	☐1	☐2	☐3
15. Mutated factor V Leiden:	☐1	☐2	☐3
16. Hyperhomocysteinemia:	☐1	☐2	☐3
Reproductive system			
17. Presence of two ovaries	☐1	☐2	☐3
18. Ovarian cyst	☐1	☐2	☐3

Diseases or disorders BEFORE this pregnancy	YES	NO	Not documented
19. Fibroid tumour	☐1	☐2	☐3
19.1 Operated on?	☐1	☐2	☐3
19.2 Treated?	☐1	☐2	☐3
Tumour			
20. Tumour?	☐1	☐2	☐3
20.1 Benign?	☐1	☐2	☐3
20.2 Location: _____			
20.3 Operated on?	☐1	☐2	☐3
Endometriosis			
21. History of endometriosis	☐1	☐2	☐3
Endocrine disorder			
22. Endocrine disorder	☐1	☐2	☐3
22.1 Specify: (hyper/hypothyroidism, delayed growth)_____			
22.2 Surgery for an endocrine disorder	☐1	☐2	☐3

B. Maternal gynecological and obstetrical history

Previous pregnancies

1. **Previous pregnancies**:

	number		number
1.1 Gravida		**1.5** Full-term children	
1.2 Para		**1.6** Preterm children (< 37 weeks)	
1.3 VIP		**1.7** Stillbirths	
1.4 Spontaneous abortions		**1.8** Perinatal deaths	

Previous deliveries

2. Previous deliveries (start with the most recent) **1st baby** □

2.X.1 **Date** DD/MM/YYYY	2.X.2 **Length** (weeks)	2.X.3 **Type** V=vaginal C=Caesarean	2.X.4 **Baby's weight** (g)	2.X.5 **Baby's length** (cm)	2.X.6 **Gestational diabetes** Yes/No	2.X.7 **Hypertensive disorders of pregnancy** Yes/No	2.X.8 **Other complications (specify)**
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	
D D / M M / Y Y Y Y					Y□ N□	Y□ N□	

Sexually transmitted infections

3. Sexually transmitted diseases: Yes: □1 No: □2

Name	3.X.1 **Yes / No**	3.X.2 **Date**
3.1 Genital herpes	Yes: □1 No: □2	Y Y Y Y
3.2 HIV (AIDS)	Yes: □1 No: □2	Y Y Y Y
3.3 Chlamydia	Yes: □1 No: □2	Y Y Y Y
3.4 Gonorrhea	Yes: □1 No: □2	Y Y Y Y
3.5 Syphilis	Yes: □1 No: □2	Y Y Y Y
3.6 Condylomas	Yes: □1 No: □2	Y Y Y Y
3.7 Ureaplasma	Yes: □1 No: □2	Y Y Y Y
3.8 Salpingitis	Yes: □1 No: □2	Y Y Y Y
3.9 Trichomonas	Yes: □1 No: □2	Y Y Y Y
3.10 Other Specify:	Yes: □1 No: □2	Y Y Y Y

C. History of current pregnancy

Current pregnancy

LNMP

1. Date of last normal menstrual period: D D / M M / Y Y Y Y

EDD

2. Expected date of delivery: D D / M M / Y Y Y Y

ADD

3. Actual date of delivery: D D / M M / Y Y Y Y

ART

4. Assisted reproductive technology: Yes: □1 No: □2 Not documented: □3

4.1 If yes, which method: _____

Prenatal visits

5. Prenatal visits: Yes: □1 No: □2 Not documented: □3

Summary table of three prenatal visits only: the first, one during the second trimester and one during the third trimester (especially if medical treatment)

	5.X.1 **Date** DD/MM/YY	5.X.2 **Weight** (kg)	**Pressure** 5.X.3 **Systolic** / 5.X.4 **Diastolic** (mm Hg)	5.X.5 **Hemo-globin** (g/L)	5.X.6 **Protein-uria** mg/24h	5.X.7 **Hematuria** Yes / No
5.1	D D / M M / Y Y Y Y		/			Y□ N□
5.2	D D / M M / Y Y Y Y		/			Y□ N□
5.3	D D / M M / Y Y Y Y		/			Y□ N□

Triple test

6. Triple test results

6.1: α-fetoprotein: _____ MoM

6.2: β-hCG: _____ MoM

Hypertension during this pregnancy

Presence

7. Presence of **hypertension during this pregnancy**: Yes: □1 No: □2

7.1 Date hypertension was identified during the pregnancy:

Date: | D | D | / | M | M | / | Y | Y | Y | Y |

7.2 Highest blood pressure recorded before medication:

7.2.1 Systolic				**mm Hg**
7.2.2 Diastolic				**mm Hg**

7.3. If yes, treated with which medication?

7.3.1 Name of the medication used at the beginning of pregnancy: _____

7.3.2 Daily dose (e.g., one 25 mg tablet 3X daily = 75 mg):

			mg

7.4. If medication, highest blood pressure recorded subsequently? (mm Hg)

7.4.1 Systolic				**mm Hg**
7.4.2 Diastolic				**mm Hg**

D. Diseases or disorders during this pregnancy

Presence of…	XX.1 Yes / No	XX.2 Trimester	XX.3 Comments
1. Gestational diabetes	Y☐ N☐		
2. Gestational diabetes treated with insulin	Y☐ N☐		
3. Oedema	Y☐ N☐		
4. Hematuria	Y☐ N☐		
5. Hematuria concomitant with urinary infection	Y☐ N☐		
6. Intrauterine growth restriction	Y☐ N☐		
7. Oligohydramnios	Y☐ N☐		
8. *Hydrops fetalis*	Y☐ N☐		
9. Infections	Y☐ N☐		
9.1 … genital	Y☐ N☐		
9.2 … urinary	Y☐ N☐		
9.3 … oropharyngeal	Y☐ N☐		
9.4 … pulmonary	Y☐ N☐		
9.5 … oral	Y☐ N☐		
9.6 … other (specify in comments)	Y☐ N☐		
10. Proteinuria (>300 mg/24 h; dipstick ≥1+)	Y☐ N☐		
11. Very high systolic pressure (>160 mm Hg)	Y☐ N☐		
12. Very high diastolic pressure (>110 mm Hg)	Y☐ N☐		
13. High serum creatinine (>105 μM)	Y☐ N☐		
14. Convulsions (eclampsia)	Y☐ N☐		
15. HELLP syndrome	Y☐ N☐		
16. Thrombocytopenia (platelets<4 x10^{10}/L)	Y☐ N☐		
17. Oliguria (< 500 mL/24h)	Y☐ N☐		
18. *Placenta previa* or low-lying placenta	Y☐ N☐		

Presence of …	XX.1 **Yes / No**	XX.2 **Trimester**	XX.3 **Comments**
19. Premature abruption of normal placenta	Y☐ N☐		
20. Absent or reversed umbilical artery end-diastolic flow by velocimetry	Y☐ N☐		
21. Other (specify in comments)	Y☐ N☐		

Hospitalization

22. Hospitalization? **Yes** ☐1 **No** ☐2

22.1 If yes, **reason:** _____

22.2 Date of admission: D D / M M / Y Y Y Y

22.3 Length (days): ☐☐☐ **days**

22.4 Other hospitalizations: _____

_____

E. Medication taken during this pregnancy

1. What medications did the patient take during the **first 20 weeks**?

1.1. Noted in the record: Yes: □1 No: □2

Non-prescription medications	Yes	No	Prescription medications	Yes	No
			1.6 Celecoxib --- Celebrex®	□1	□2
1.2. Ibuprofen (Advil®)	□1	□2	**1.7** Diclofenac --- Voltaren ®	□1	□2
1.3 Naproxen (Aleve® or Naprosyn®)	□1	□2	**1.8** Etodolac --- Lodine®	□1	□2
1.4 Aspirin (Bayer®)	□1	□2	**1.9** Fenoprofen --- Nalfon®	□1	□2
1.5 Acetaminophen (Tylenol®)	□1	□2	**1.10** Indomethacin --- Indocin®	□1	□2
			1.11 Ketoprofen-Orudis®, Oruvail®	□1	□2
			1.12 Ketoralac --- Toradol®	□1	□2
			1.13 Oxaprozine ---Daypro®…	□1	□2
			1.14 Nabumetone --- Relafen®	□1	□2
			1.15 Sulindac --- Clinoril®	□1	□2
			1.16 Tolmetin --- Tolectin®	□1	□2
			1.17 Rofecoxib --- Vioxx®	□1	□2

1.18. Other medications, supplements, natural supplements Yes □1 No □2

1.18.1 If yes, specify_____

2. What medications did the patient take during the **last 20 weeks**?

2.1 Noted in the record: Yes: □1 No: □2

Non-prescription medications	Yes	No	Prescription medications	Yes	No
			2.6. Celecoxib --- Celebrex®	□1	□2
2.2 Ibuprofen (Advil®)	□1	□2	**2.7** Diclofenac --- Voltaren ®	□1	□2
2.3 Naproxen (Aleve® ou Naprosyn®)	□1	□2	**2.8** Etodolac --- Lodine®	□1	□2
2.4 Aspirin (Bayer®)	□1	□2	**2.9** Fenoprofen --- Nalfon®	□1	□2
2.5 Acetaminophen (Tylenol®)	□1	□2	**2.10**Indomethacin --- Indocin®	□1	□2
			2.11 Ketoprofen-Orudis®, Oruvail®	□1	□2
			2.12 Ketoralac --- Toradol®	□1	□2
			2.13 Oxaprozine ---Daypro®…	□1	□2
			2.14 Nabumetone --- Relafen®	□1	□2
			2.15 Sulindac --- Clinoril®	□1	□2
			2.16 Tolmetin --- Tolectin®	□1	□2
			2.17 Rofecoxib --- Vioxx®	□1	□2

2.18 Other medications, supplements, natural supplements Yes □1 No □2

2.18.1 If yes, specify_____

F. Delivery

Anthropometric data

			–											

Patient identification **Code**

Human biological specimen bank for the study of complications of pregnancy, maternal-fetal exchanges and their subsequent consequences

1. Patient's **weight** on admission for delivery: | | | | . | | **kg**

Gestational age

2. Gestational age on admission for delivery: 2.1 | | | **weeks** 2.2 | | **days**

3. Gestational age at delivery: 3.1 | | | **weeks** 3.2 | | **days**

3.3 Confirmed by LMP: □1 ultrasound: □2 both: □3

Blood pressure

4. Blood pressure

4.1 On **admission** for delivery:

4.1.1 Systolic				**mm Hg**
4.1.2 Diastolic				**mm Hg**

4.2. HIGHEST pressure DURING labour:

4.2.1 Systolic				**mm Hg**
4.2.2 Diastolic				**mm Hg**

4.2.3 At what time (put hh on a 24-hour clock):

D	D	/	M	M	/	Y	Y	Y	Y	/	h	h	/	m	m

4.3. HIGHEST pressure AFTER delivery:

4.3.1 Systolic				**mm Hg**
4.3.2 Diastolic				**mm Hg**

4.3.3 At what time (put hh on a 24-hour clock):

D D / M M / Y Y Y Y / h h / m m

Proteinuria

5.1. On **admission** for delivery: Yes: □1 No: □2 Not documented: □3

5.1.1 If yes, result (dipstick): 1+:□1 2+:□2 3+:□3

5.1.2 If yes, result (24 hour): | | | | **mg/24 h**

5.2. **During labour**: Yes: □1 No: □2 Not documented: □3

5.2.1 If yes, result (dipstick): 1+ :□1 2+ :□2 3+ :□3

5.2.2 If yes, result (24 hour): | | | | **mg/24 h**

5.3.**After delivery**: Yes: □1 No: □2 Not documented: □3

5.3.1 If yes, result (dipstick): 1+ :□1 2+ :□2 3+ :□3

5.3.2 If yes, result (24 hour): | | | | **mg/24 h**

Diseases, disorders and treatments	**YES**	**NO**	**Not documented**
6. Oedema:	□1	□2	□3
7. Pulmonary oedema:	□1	□2	□3
8. Eclampsia:	□1	□2	□3
9. Preeclampsia:	□1	□2	□3
10. Kidney failure:	□1	□2	□3
11. HELLP syndrome:	□1	□2	□3

Diseases, disorders and treatments

YES NO Not documented

12. Magnesium sulfate therapy: ☐1 ☐2 ☐3

12.1	If yes, maximum **length** of treatment		**days**
12.2	If yes, **dose received**		**mg**
12.3	If yes, **maximum magnesemia**		**µM**

13. Corticosteroid therapy: ☐1 ☐2 ☐3

		13.X.1 **Date**	13.X.2 **Dose** (mg)
13.1	If yes, first dose	D D / M M / Y Y Y Y	
13.2	If yes, last dose	D D / M M / Y Y Y Y	

14. Tocolytic therapy: ☐1 ☐2 ☐3

14.1	If yes, name of treatment		
14.2	If yes, maximum **length** of treatment		**days**
14.3	If yes, **dose received**		**mg**

15. Antibiotics: ☐1 ☐2 ☐3

15.1	If yes, name of treatment	

16. Other medications: ☐1 ☐2 ☐3

16.1	If yes, name of treatment	

Delivery

17. Type of delivery vaginal □1 Caesarean □2

17.1 If Caesarean, elective □1 emergency □2

17.2 If Caesarean, reasons: _____

18. Labour spontaneous □1 medically induced □2 none □3

19. Length of labour | | | **hours**

20. Anesthesia Yes □1 No □2 Not documented □3

20.1 If yes, specify: _____

Diseases, disorders and treatments

	YES	NO	Not documented
21. Fever (> 38.3 °C)	□1	□2	□3

21.1 If yes, at what time (put hh on a 24-hour clock):

D	D	/	M	M	/	Y	Y	Y	Y	/	h	h	/	m	m

22. Significant blood loss (> 500 mL):	□1	□2	□3
23. Funisitis in the mother:	□1	□2	□3
24. Endometritis in the mother:	□1	□2	□3
25. Post-partum **hemorrhage** in the mother:	□1	□2	□3

22.1. If yes, amount recorded: | | | | | **mL**

25.1. If yes, medication or treatment: _____

26. Other: _____

G. Information about the baby

Birth

1. **Baby's date of birth:** D D / M M / Y Y Y Y h h : m m
2. **Place of birth**: _____
3. **Sex:** M: □1 F: □2
4. **Type of birth:** singleton: □1 twins: □2 triplets: □3
5. **Apgar score**: **1 min** 5.1 | **5 min** 5.2 | **10 min** 5.3

Baby's anthropometric data

6.1	**Weight**					**g**
6.2	**Length**					**cm**
6.3	**Head circumference**					**cm**
6.4	**Chest circumference**					**cm**

7. **Weight of the placenta**: **g**

7.1 Additional information (e.g., anomalies): _____

Clinical data

8. **Stillbirth:** Yes: □1 No: □2
9. Admission to the **intensive care** unit: Yes: □1 No: □2
10. **Septicemia**: Yes: □1 No: □2
11. **Pneumonia**: Yes: □1 No: □2
12. **Necrotic enteritis**: Yes: □1 No: □2
13. **Hemorrhage**: Yes: □1 No: □2
14. **Jaundice**: Yes: □1 No: □2
15. **Other complications**: _____
16. Baby **breastfed** in hospital? Yes: □1 No: □2