Calcium and Vitamin D Intake During Pregnancy and Postpartum in the Alberta Pregnancy Outcomes and Nutrition (APrON) Study

by

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Abstract

Calcium and vitamin D are important in maintaining a healthy pregnancy. Low intake/status has been associated with preeclampsia, preterm delivery, lower birth weight, poor fetal skeletal growth, reduced bone mass, and excessive maternal bone loss. Rarely have these nutrients been examined together in intake studies during pregnancy and postpartum. The primary research objective was to describe calcium and vitamin D intake across pregnancy and 3 months postpartum in a large cohort of women (n~1054) in Alberta. The secondary objective was to identify the sources of calcium and vitamin D in the diet of the cohort. The cohort consisted of older women (mean: 31 ± 5 years old) with a healthy BMI (24.2 ± 4.7), and high socioeconomic status. Diet (24-hour recall) and supplement intakes were collected up to 3 times (once during each trimester) and 3 months postpartum and analyzed using Food Processor and a supplement database. Estimated calcium intake came mainly from diet whereas vitamin D mainly came from supplements. Estimated mean vitamin D supplement intake in this cohort was higher than reported in the literature. Estimated mean calcium intake (diet, supplement, and total) increased with each trimester, however, was significantly lower at 3 months postpartum ($p < 0.05$). Estimated mean vitamin D from food/beverages intake did not change through pregnancy and postpartum, but supplement and hence total intake increased significantly with each trimester and at 3 months postpartum ($p < 0.05$). The cohort met the EAR for calcium with diet only, but 20% of women did not meet the EAR at 3 months postpartum. At all time points, women did not meet the EAR for vitamin D with diet alone and relied on supplement intake to meet recommendations. Despite this, 23% of women in the first trimester and postpartum still did not meet the EAR for vitamin D. Without 'adequate' vitamin D intake, calcium cannot be absorbed as well. In the cohort, 19-34% of women had either intake below the EAR for calcium or vitamin D intake below the EAR, placing them at risk. Of women that exclusively breast fed, 71% met the EAR for total calcium and these women were 2.9 times (95% CI: 1.67-4.8, $p < 0.001$) more likely to meet the EAR than those that exclusively formula fed. Of women that exclusively breast fed, 81% met the EAR for total vitamin D and these women were 2.3 times (95% CI: 1.37-4.0, $p = 0.002$) times more likely to meet the EAR than those that exclusively formula fed. There was a moderate ($r = 0.47$, $p < 0.001$) correlation between caloric intake and estimated calcium intake and no correlation between caloric intake and estimated vitamin D intake ($r = 0.09$, $p < 0.001$). Supplement users (SU) had significantly higher estimated mean total vitamin D intake (921.8 ± 126.2 IU) than non-supplement users (NSU) (212.1 ± 16 IU) at all four time points ($p < 0.05$). Aerobic exercise, planned pregnancy, ethnicity, and weight change status were identified as predictors of calcium intake and aerobic exercise, marital status, and income were found to significantly affect a woman's ability to meet the EAR for vitamin D. While calcium fortified foods and beverages minimally contributed to estimated dietary intake, vitamin D fortified foods/beverages majorly contributed to intake. As high as 40% of estimated vitamin D in the diet came from vitamin D fortified cow's milk and 9% came from other fortified sources such as juices, plant-based beverages, and margarine. Medium to high milk drinkers (> 250 mL/day) were significantly ($p < 0.001$) more likely to meet the EAR than women that drank no milk. In conclusion, the women in this cohort met calcium recommendations with diet but depended on supplement intake to meet vitamin D recommendations. Milk drinkers were more likely to meet the recommendations than non-milk drinkers. Women may be at greater risk for suboptimal calcium status due to decreased absorption from low vitamin D intake. The postpartum period was a time where estimated calcium intake decreased and further education emphasizing the importance of increasing both nutrient intakes may need to be done to ensure women are meeting recommendations for bone loss prevention. Future studies may include incorporating vitamin D status as well as examining the relationship between dietary intake and maternal and infant health outcomes.

Preface

This thesis is an original work by Amy R. Weinberg. The research project of which this thesis is a part of received research ethics approval from the University of Alberta Research Ethics Board, "Alberta Pregnancy Outcome and Nutrition (APrON)", No. 00002954, April 3, 2009.

Dedication

This thesis is dedicated to the important people in my life, whom were a constant source of support and encouragement from a distance through the challenges of graduate school. To Sarah, my best friend since I was eight years old and my sister, Lori, I cannot thank you two enough. I also want to dedicate this work to my mom and dad who have always believed in me, encouraged me to work hard, and taught me never to give up. To my son, Ian, who was born amidst me completing this degree, whom I love with all my heart and soul. He has been a constant source of happiness and joy in my life. To my grandparents, (Bubbie, Pop Pop, and Grandpa) who have passed away whom I know would have been so proud of me reaching this milestone in my life. And lastly, to my cat Frankie, who spent countless hours on my lap as I wrote this dissertation. I am grateful for all of you so much!

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List of Abbreviations

25(OH)D-	Calcidiol
$1,25(OH)_2D$-	Calcitriol
μg-	Micrograms
AHS-	Alberta Health Services
AI-	Adequate Intake
APrON-	Alberta Pregnancy Outcomes and Nutrition
BID-	Twice a day
BMI-	Body Mass Index
C.-	Cups
Ca-	Calcium
CaR-	Calcium receptor
CCHS-	Canadian Community Health Survey
CI-	Confidence Interval
CPS-	Canadian Pediatric Society
CQ-	Calcium Questionnaire
CVD-	Cardiovascular disease
D_2-	Ergocalciferol

D_3-	Cholecalciferol
DHQ-	Diet History Questionnaire
DRI-	Dietary Reference Intake
EAR-	Estimated Average Requirement
GDM-	Gestational Diabetes
IBD-	Inflammatory Bowel Disease
IOM-	Institute of Medicine
Kcal-	Kilocalories
LNHPD-	Licensed Natural Health Products Database
Mg-	Milligram
Mmol/L-	Millimoles per liter
MUFA-	Monounsaturated fatty acid
NHANES-	National Health and Nutrition Examination Surveys (USA)
NHPs-	Natural Health Products
NIH-	National Institutes of Health
Nmol/L-	Nanomoles per liter
NNR-	Nordic Nutrition Recommendations
NSU-	Non-supplement user

OR-	Odds ratio
Oz.-	Ounces
PTH-	Parathyroid hormone
PTHR-	Parathyroid receptor
PUFA-	polyunsaturated fatty acid
RA-	Rheumatoid arthritis
RCT-	Randomized control trial
RD-	Registered Dietitian
RDA-	Recommended Dietary Allowance
SCQ-	Short Calcium Questionnaire
SD-	Standard Deviation
SIQ-	Supplement Intake Questionnaire
SU-	Supplement user
T1DM-	Type 1 Diabetes Mellitus
Tbs.-	Tablespoon
TP-A-	Time point A (1st trimester)
TP-B-	Time point B (2nd trimester)
TP-C-	Time point C (3rd trimester)

TP-E-	Time point E (3 months postpartum)
UK-	United Kingdom
UL-	Tolerable Upper Intake Level
VDR-	Vitamin D receptor
Vit D	Vitamin D
WCHRI -	Women and Children's Health Research Institute
WHO-	World Health Organization

Chapter 1: Introduction and Literature Review

1. Introduction

1.1 Calcium general background

Calcium is the most abundant mineral in the human body and an important nutrient of the human skeletal structure. Approximately 99% of calcium remains stored in teeth and bones while the other 1% remains in circulation, muscle, and the interstitial spaces. Other than its role in bone health, calcium aids in muscle contractions, the release of neurotransmitters, cardiac electro activity, as well as vasoconstriction and dilation of blood vessels.

1.1.1 Dietary sources of calcium

According to Health Canada, the United States' National Institutes of Health (NIH), and Harville et al. (2004), dairy products such as milk, yogurt, and cheese are major calcium contributors to the North American diet. However, over the last century, there has been a decrease in milk consumption and therefore a large contributor to overall dietary calcium intake (Thomas and Weisman, 2006). Non-dairy sources of calcium for Canadians include dark green vegetables such as kale, broccoli, and spinach and canned fish with soft bones such as sardines and salmon. The calcium content of tap and commercially bottled water depends greatly on hardness and geographical location (Ross et al., 2011). In a study of tap water conducted in 21 major cities in North American, calcium content did not contribute significantly to overall dietary intake (range: 18-52mg/L). With the exception of mineral water (100 ± 125 mg/L), the calcium profiles of tap and bottled water were similar (Azoulay et al., 2001).

1.1.2 Calcium fortified foods and beverages

In addition to naturally occurring calcium, several foods and beverages are fortified in Canada, including plant-based beverages such as soy, rice, and almond beverages, fruit juices, tofu, and ready-to-eat cereals (Vitamin D and Calcium: Updated Dietary Reference Intakes, 2012). **Table 1.1** shows the calcium content of various foods and beverages. Fortified plant-based beverages contain as much calcium per serving as the cow's milk they are replacing.

Table 1.1: Calcium content of common foods and beverages

Food	**Serving**	**Calcium content (mg)**
Plain yogurt	8 oz. (235.6 mL)	415 mg
Mozzarella cheese	1.5 oz. (42.5 g)	333 mg
Sardines (packed in oil) with bones	3 oz. (88.7 g)	325 mg
Cheddar cheese	1.5 oz. (42.5 g)	307 mg
Rice beverage, fortified*	8 oz. (235.6 mL)	302 mg
Almond beverage, fortified*	8 oz. (235.6 mL)	296 mg
Milk (averaging whole, 2%, skim)	8 oz. (235.6 mL)	289 mg
Calcium fortified soy beverage	8 oz. (235.6 mL)	299 mg
Tofu, firm, fortified	½ c.	253 mg
Calcium fortified orange juice*	6 oz. (176.7 mL)	219 mg
Salmon (canned with bones)	3 oz. (88.7 g)	181 mg
Cottage cheese (1% milkfat)	8 oz. (235.6 mL)	138 mg
Kale, raw	1 c.	100 mg
Ready-to-eat fortified cereals	1 c.	100-1000 mg
Sour cream, reduced fat	2 tbs.	31mg
Whole wheat bread	1 slice	30 mg
Broccoli, raw	½ c.	21 mg

Cream cheese, regular	1 tbs.	14 mg

Source: http://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/ www.hc-sc.gc.ca*

1.1.3 Calcium supplementation

The two most common forms of calcium supplements in North American are calcium citrate and carbonate (Straub, 2007). Calcium carbonate is the most common form in prenatal vitamins and single supplements contain either carbonate or citrate. Since calcium carbonate requires stomach acid to aid in absorption, it needs to be consumed with meals while calcium citrate does not. Prenatal vitamin supplements in Canada contain approximately 200-400 mg of calcium per tablet. **Appendix A** shows the calcium content of common prenatal vitamins in Canada.

1.1.4 Interferences with calcium absorption

Even though a serving of green vegetables such as broccoli, kale, and bok choy can contain more calcium than some dairy and fortified foods **(Table 1.1)**, they contain the compound oxalic acid which inhibits calcium absorption. Other foods high in oxalic acid include spinach, collard greens, sweet potato, and legumes. Other foods that contain phytic acid which also decreases calcium absorption include whole grains, bran, nuts, seeds, spinach, and soy protein isolate often used as stabilizers and emulsifiers in foods (Dietary Supplement Fact Sheet for Healthcare Professionals: Calcium- NIH, 2012). Caffeine decreases calcium absorption as well but not significantly as previously studies indicated (Heaney, 2002; Massey and Whiting, 1993).

1.2 Vitamin D general background

Vitamin D is a fat soluble vitamin that has varying functions in the body. Vitamin D plays a role in bone health by promoting calcium absorption in the gut and maintaining serum calcium and phosphorus homeostasis to enable normal bone mineralization. It is also needed for bone growth and remodeling by osteoblasts and osteoclasts.

1.2.1 Vitamin D and disease prevention

Other than bone health, vitamin D is involved in several other biological functions including the reduction of inflammation, neuromuscular and immune function, and the modulation of cell growth and genetic coding that regulates cell proliferation, differentiation, and apoptosis (reviewed by Kaludjerovic and Vieth, 2010; Hypönnen, 2011). More recently, emerging evidence supports that poor vitamin D status may be associated with an increased risk of cardiovascular disease (CVD) as well as autoimmune disorders including type 1 diabetes mellitus (T1DM), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD) (reviewed by Kaludjerovic and Vieth, 2010; Ross et al., 2011). Large cross-sectional studies of both men and women have shown an increased risk of cardiac disease with plasma vitamin D concentrations anywhere from < 25 nmol/L to 50 nmol/L (insufficient to deficient status as defined by the IOM) (Kim et al., 2008; Kendrick et al., 2009). In fact, in a study looking at the prevalence of CVD in the National Health And Nutrition Examination Surveys (NHANES) 2001-2004, participants with serum 25(OH)D concentrations less than 50 nmol/L had an adjusted odds ratio of 1.41 (95% CI: 1.08-1.84, p= 0.015) for coronary heart disease and 1.73 (95% CI:1.03-2.91, p= 0.029) for congestive heart failure when compared to participants with serum 25(OH)D concentrations of ≥ 75 nmol/L (Kim et al. 2008). A CVD double-blinded RCT of generally healthy post-menopausal women found no significant differences between reported vitamin D supplement use (62.5 μg (2500 IU)/day for four months) and non-use for the reduction of cardiac risk factors for CVD such as arterial stiffness, inflammation, and altered arterial function (Gepner et al., 2012). While one other RCT study found similar results, they supplemented with a dose of 5 μg (200 IU) twice per day over seven years which has been reported in the literature to be too low to detect change in CVD risk (Hsia et al., 2007).

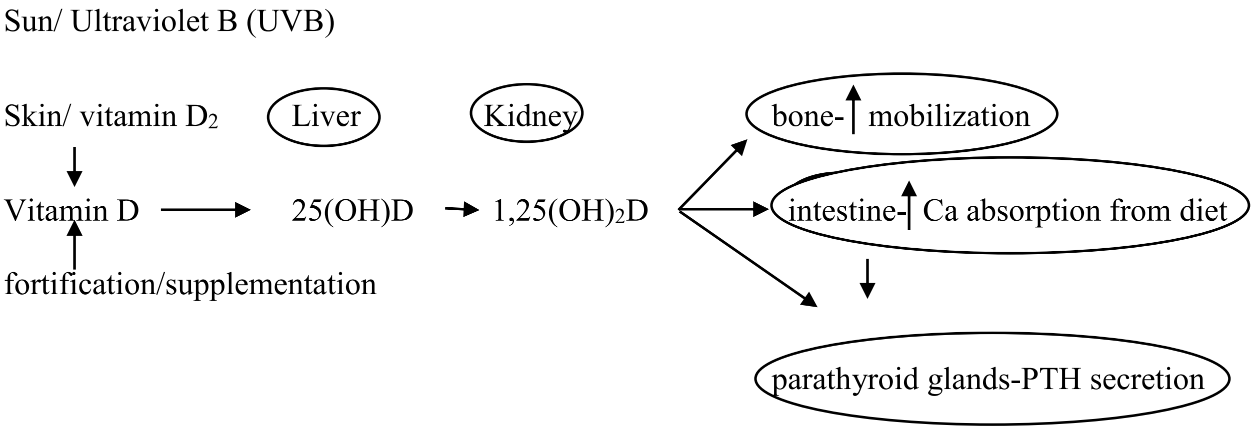
The incidence of gestational diabetes (GDM) in Alberta is rising which has been thought to be related to increased maternal age and obesity (Nerenberg et al. 2013). According to Nerenberg et al. (2013) which obtained data from the Alberta Perinatal Health Program which collects information on all deliveries in the province, from 2001-2009, the incidence of GDM increased significantly ($p < 0.01$) from 3.1% to 4.6%. A recent critical literature review found that lower vitamin D status was associated with the development of GDM even after controlling for confounding variables for vitamin D intake and GDM (Agarwal et al., 2016). A Canadian prospective cohort study of 655 women from the review found that those that developed GDM had significantly lower serum 25(OH)D concentrations at baseline during the first trimester (57.5 ± 17.2 nmol/L) than women that were euglycemic (63.5. ± 18.9 nmol/L, $p = 0.03$). Those women with lower first trimester vitamin D status were 1.48 times (95% CI: 1.03-2.12, $p = 0.04$) more likely to develop GDM during pregnancy (Lacroix et al., 2014). Arnold et al. (2015) also found serum 25(OH)D concentrations to be significantly lower for women with GDM than those euglycemic controls (62.3 ± 21.8 nmol/L vs. 73.25 ± 20.8 nmol/L, $p = 0.01$). Women whose serum 25(OH)D were in the deficient range defined as < 50 nmol/L for this study had a 1.95 times greater risk of developing GDM than women with sufficient status (< 75 nmol/L) (95% CI: 1.16-3.29, $p = 0.012$) (Arnold et al., 2015). The results of RCTs have been more inconclusive (Agarwal et al., 2016).

Preeclampsia, which effects approximately 2-8% of first time mothers and 1.3% of pregnant women in Alberta, has also been associated with low maternal vitamin D status (Nerenberg et al., 2013). A meta-analysis of observational studies found that mothers with higher concentrations of serum 25(OH)D had decreased odds of developing preeclampsia compared to those with lower status; pooled OR 0.52 (95% CI: 0.30-0.89, $p= 0.02$). A few placebo controlled studies as well as one blinded trial of vitamin D supplementation which ranged from 10 µg (400 IU)/day for the control group to 50-100 µg (2000-4000 IU)/day for the treatment groups, showed a reduced odds of preeclampsia of the treatment group than the control group, with a pooled OR 0.62 (95% CI: 0.52-0.83, $p= 0.001$) (Hypönnen et al., 2013).

1.2.2 Forms of vitamin D

The two most common forms of vitamin D in supplements and the diet are ergocalciferol (D_2) and cholecalciferol (D_3). They can be obtained from food, supplements, as well as synthesized cutaneously from previtamin D activated by sun exposure. These forms must be converted by the liver to calcidiol (25(OH)D), which is then converted to its active form, calcitriol ($1,25(OH)_2D$) by the kidneys **(Figure 1.1)**.

Figure 1.1: Conversion of vitamin D to its active form in the body



Adapted from (www.mayomedicallaboratories.com)

1.2.3 Naturally occurring dietary sources of vitamin D

Rich sources of vitamin D include the flesh of fatty fish such as tuna, mackerel, and salmon as well as fish liver oils commonly consumed in Scandinavian countries, Ireland, and Scotland. Other foods containing small amounts of vitamin D_3 include beef liver, cheese, and egg yolks. Vitamin D_2 can be found in variable amounts in mushrooms as well.

1.2.4 Vitamin D fortified foods and beverages

In Canada, vitamin D fortification is required under the Food and Drug Act to the following products: margarine, meal replacement/nutritional supplements, milk which includes cow and goat as well as evaporated, condensed, or powder derivatives. Milk and margarine must contain 0.88-1 µg (35-40 IU)/100 mL and ≥ 13.25 µg (530 IU)/100 g, respectively. Other voluntarily fortified foods and beverages include yogurt, ready-to-eat cereals, plant-based beverages, and orange juice. **Table 1.2** shows examples of naturally occurring and fortified sources of vitamin D.

Table 1.2: Vitamin D content of common foods and beverages

Food source	Serving size	Vitamin D content
Cod liver oil	1 tbs. (15 g)	34 µg (1360 IU)
Salmon	3 oz. (88.7 g)	11.18 µg (447 IU)
Canned tuna, drained	3 oz. (88.7 g)	3.85 µg (154 IU)
Milk, fortified (non-fat, reduced fat, whole)*	1 c. (235.6 mL)	2.57 µg (102.8 IU)
Orange juice, fortified*	1 c. (235.6 mL)	2.36 µg (94.4 IU)

Rice beverage, fortified*	1 c. (235.6 mL)	2.07 µg (82.8 IU)
Soy beverage, fortified*	1 c. (235.6 mL)	2.06 µg (82.6 IU)
Margarine, stick, fortified*	1 tbs. (15 g)	1.99 µg (79.6 IU)
Almond beverage, fortified*	1 c. (235.6 mL)	1.98 µg (79.2 IU)
Yogurt (plain), fortified*	6 oz. (177.4 g)	1.8 µg (72 IU)
Sardines, canned in oil	2 pieces	1.15 µg (46 IU)
Beef liver	3 oz. (88.7 g)	1.05 µg (42 IU)
Egg	1 large	1.03 µg (41 IU)
Swiss cheese	1 oz. (29.6 g)	0.15 µg (6 IU)

Source: http://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/
www.hc-sc.gc.ca*

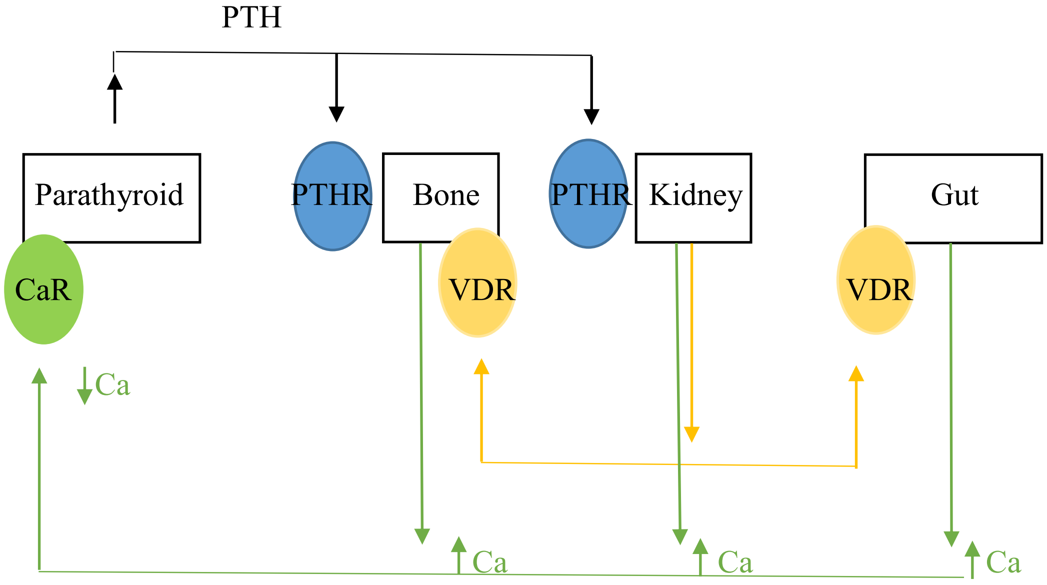
Other products made from dairy such as cheese and ice cream are generally not fortified. The fortification of milk was initiated in the 1920s and 1930s as a response to the widening presence of rickets in children caused by vitamin D deficiency. However, deficiency, can extend into adulthood leading to osteopenia and osteoporosis as humans age. Since limited foods contain vitamin D in the Western diet, vitamin D fortified foods and beverages can significantly contribute to meeting dietary guidelines (Institute of Medicine, Food and Nutrition Board and Dietary Reference Intakes for Calcium and Vitamin D, 2010). However, fortification only aids in meeting nutritional needs if people are consuming these products. Whether or not mandatory fortification of margarine and milk as well as encouraging naturally occurring vitamin D products is enough to meet current nutrient recommendations during pregnancy and postpartum is not clear.

1.3 Metabolic relationship between calcium and vitamin D

Vitamin D plays a role in maintaining calcium homeostasis in the body whether it be calcium obtained from the gut, kidneys, or bones. **Figure 1.2** illustrates how the body regulates calcium. When serum calcium concentrations are low, calcium receptors (CaR) become activated which signals the parathyroid gland to excrete parathyroid hormone (PTH), causing parathyroid receptors (PTHR) in the bone to increase resorption as well as increase reabsorption by the distal convoluted tubule in the nephrons of the kidneys. This causes a greater production of calcitriol, which acts on vitamin D receptors (VDR) in the gut to increase calcium absorption and bone resorption. Regardless of whether calcium is obtained by supplement or food, the small intestines only absorbs approximately 30% of the total amount consumed in a healthy person. The majority of absorption occurs through the calcitriol mechanism rather than passive diffusion.

With insufficient vitamin D status due to low dietary intake or inadequate sun exposure, calcium absorption in the gut decreases (Need et al., 2008; reviewed by Christakos et al., 2014). An osteoporosis study of 319 men and women with a serum 25(OH)D concentrations of $\leq$ 40 nmol/L which was noted to be the concentration where 1,25(OH)$_2$D (calcitriol) was preserved due to secondary hyperparathyroidism found that calcium absorption was maintained until serum 25(OH)D concentrations fell below 11 nmol/L (Need et al., 2008). However, this study was not done during pregnancy and lactation and the consequences of poor status of vitamin D on calcium absorption are not known. From cross-sectional studies, there was also a positive association between calcium absorption and serum 1,25(OH)$_2$D concentration (the active form of vitamin D) suggesting less active form production from available 25 (OH)D substrate produced by the liver (reviewed by Christakos et al., 2014).

Figure 1.2: Calcium homeostasis



(Adapted from Peacock, 2010)

1.4 Calcium and vitamin D recommendations

The Dietary Reference Intake (DRI), are a "comprehensive set of nutrient reference values for healthy populations that can be used for assessing and planning diets" (Dietary Reference Intakes- Health Canada, 2013). The recommendations are reviewed and modified if new evidence-based research warrants a change. The DRI encompass three types of nutrient reference values for calcium and vitamin D: Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), and Tolerable Upper Intake Level (UL). The RDA for an age and sex category is set at a level that would satisfy the requirements of 97-98% of healthy persons. One should use caution when describing a person's nutrient intake as inadequate when it is below the RDA since he/she may be meeting his/her own personal needs. The EAR, on the other hand, represents the amount of a nutrient needed to meet the requirement of 50% of all healthy persons in a given population. The EAR can be used to assess dietary inadequacy in a population but should not be used as a recommendation of intake since it only satisfies the requirements of half the population. Calcium and vitamin D have an UL, which is the highest recommended dose to be consumed daily without posing any adverse health effects.

The recommendations for both calcium and vitamin D are based on bone health research as an indicator of adequacy (Dietary Reference Intakes: Calcium and Vitamin D, 2011) **(Table 1.3)**. The EAR and RDA for calcium for a female ages 19-50 years old is 800 mg/day and 1000 mg/day, respectively. The EAR and RDA do not increase in pregnancy or lactation because calcium absorption increases from 33-36% pre-pregnancy to 54-62% during pregnancy to compensate for the increased demand from the fetus and ultimately the breast fed infant (reviewed by Kaludjerovic and Vieth, 2010; reviewed by Hacker, et al., 2012). Exceeding the UL of 2500 mg/day is more common with supplement use and has been associated with increased risk of kidney stones in non-pregnant women (Curhan et al., 1997). The systematic reviews and meta-analyses of calcium supplementation and the increased risk of cardiovascular diseases has remained inconclusive. RCTs in this area, which are the gold standard in research, have several shortcomings including not having calcium intake as a primary pre-specified primary outcome, the number of adverse cardiac events were not great enough to draw any clinically relevant conclusions, studies were not double-blinded, and there were no significant increases in cardiovascular disease events between those taking a calcium supplements and those taking a placebo (Wang et al., 2012). In longitudinal prospective cohort studies, self-reported data such as the classification of cardiac events and not controlling for confounding variables limited the reliability of the studies (reviewed by Heaney et al., 2012).

The EAR and RDA for vitamin D for a female ages 19-50 years old is 10 µg (400 IU) and 15 µg (600 IU)/day, respectively. Although vitamin D can be endogenously synthesized, the current recommendations reflect intake derived from dietary sources assuming minimal sun exposure. The UL is 100 µg (4000 IU)/day. Similar to calcium, exceeding the UL is more likely to occur with supplementation. Symptoms of vitamin D toxicity include anorexia, weight loss, polyuria, and cardiac arrhythmias. A more long term effect for healthy adults consuming in excess of 250-1000 µg (10,000-40,000 IU/day) for greater than five months includes hypercalcemia which can lead vascular and soft tissue calcification damaging the heart, kidneys, and blood vessels (Heaney et al., 2003; Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D, 2010). Only animal studies have shown vitamin D_2 to be less toxic than D_3 when given at the same high dose; however due to ethical issues, this cannot be tested in human trials (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D, 2010).

Table 1.3: Dietary Reference Intakes

Nutrient	**EAR Preg.** 14-18 years old	**EAR Preg.** 19-50 years old	**RDA Preg.** 14-18 years old	**RDA Preg.** 19-50 years old	**UL Preg.** 14-18 years old	**UL Preg.** 19-50 years old	**RDA Lact.** 14-18 years old	**RDA Lact.** 19-50 years old
Ca	1000 mg	800 mg	1300 mg	1000 mg	3000 mg	2500 mg	1300 mg	1000 mg
Vitamin D	10 µg (400 IU)	10 µg (400 IU)	15 µg (600 IU)	15 µg (600 IU)	100 µg (4000 IU)	100 µg (4000 IU)	15 µg (600 IU)	15 µg (600 IU)

(Source: http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/)

1.5 Calcium and vitamin D during pregnancy and postpartum

1.5.1 Significance of calcium and vitamin D during pregnancy and postpartum

Adequate dietary quality during pregnancy is vital to the mother's health status and the fetus's growth and development. During the first trimester, adequate nutrition affects organ development, differentiation, and fetal growth, and in later pregnancy, it continues to affect growth as well as brain development (Shifas-Riman et al., 2006; Rodríguez-Bernal et al., 2010). A maternal diet insufficient in calcium can increase the risk for gestational and long term complications such as preeclampsia, preterm delivery, and excessive bone loss but has limited direct effects on the developing infant as maternal stores (bones) are used to ensure the needs for fetal development are met (reviewed by Hacker et al., 2012; reviewed by Olausson et al., 2012). Only when intake was less than 500 mg/day, despite the compensatory measure of increased intestinal absorption with low intake, were maternal and fetal needs not likely met (reviewed by Hacker et al., 2012).

A diet insufficient in vitamin D as measured by low serum 25(OH)D has been associated with preeclampsia, GDM, poor fetal skeleton growth, reduced gestation, lower birth weight, and reduced bone mass throughout life (Haugen et al., 2008; Derbyshire et al., 2009; reviewed by Hyppönen, 2011, Lacroix et al., 2014). Current studies on the fetal environment suggests that the vitamin D status of the mother can possibly affect immune system functioning later in life of the offspring, making him/her more prone to diseases like T1DM, asthma, and allergies as stated prior (Valjakainen, 2010; reviewed by Hyppönen, 2011).

The importance of calcium and vitamin D for maternal and infant health continues after birth. Similar to Derbyshire et al. (2009), Mannion et al. (2007) found that women had difficulties meeting the RDA for calcium during the postpartum period. Only women who reported consuming milk had estimated mean intakes above the EAR and RDA at 1379 mg/day compared to non-milk drinkers at 895 mg/day. In this study, both groups of women were able to meet the EAR and RDA when they reported taking supplements. Regardless of intake, lactating women sequester approximately 250-400 mg/day of calcium in breast milk which is derived from maternal bone and to a lesser extent from dietary intake (Kalkwarf, 1999; Olausson et al., 2012). From the amount of calcium available in breast milk, it is estimated that 55-60% of that is absorbed by the infant (Ross et al., 2011). When calcium was supplemented during lactation, there was no effect on the maternal bone density of various sites in the body. In these studies, once weaning began, bone density was found to return to its pre-pregnancy state even in women with low dietary intake, suggesting that absorption changed (Kalkwarf, 1999; Ross et al., 2011). Regardless, it is generally assumed that dietary intake still is important in obtaining optimal bone mass prior to pregnancy, for the prevention of bone loss above and beyond the normal loss during pregnancy as well as lactation, and having optimal bone mass maintained throughout adulthood.

There is a direct relationship between dietary intake of vitamin D and status. Deficient status has been associated with rickets in infants and osteomalacia in adults, and inadequate status has been associated with the inability to support normal bone health in healthy adults (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D, 2010). It has been suggested that the current DRI are insufficient to support the vitamin D needs of the mother, and women may need to consume at least 100 µg/day (4000 IU)/day to maintain both their own as well as their infant's status during pregnancy (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D, 2010). In a small RCT of exclusively breast feeding women, they were provided with either 40 µg (1600 IU)/day of vitamin D_2 in addition to a multivitamin containing 10 µg (400 IU) D_3 or 90 µg (3600 IU)/day of vitamin D_2 in addition to 10µg (400 IU) (Hollis and Wagner, 2004). They found no adverse toxic effects in the mothers when vitamin D was given at 10 times the RDA (which was 10µg at the time) for a period of three months, and serum calcium concentration, urinary calcium excretion, and 25(OH)D_2 concentrations never exceeded the normal reference range. In this study, they reported a significant increase in 25(OH)D_2, 25(OH)D_3, and 25(OH)D_2 in the infants at four months as compared to baseline at one month old. Greater increases were observed in the infants whose mothers were in the 100 µg/day (4000 IU) group (Hollis and Wagner, 2004). Two other studies found that with a maternal intake of 17.5 µg (700 IU) and 25 µg (1000 IU)/day, respectively, infant 25(OH)D_2 concentration declined (Ala-Houhala, 1985; Greer and Marshall, 1989). Based on these RCTs, one might concluded that the current RDI are too low to support an optimal vitamin D status which would be defined as the risk reduction of adverse outcomes previously mentioned.

1.5.2 Effect of foods/beverages in the diet on calcium and vitamin D absorption

Compounds in foods and beverages can affect intestinal absorption of calcium and vitamin D. Caffeine consumption has been cited to have a negative influence on calcium absorption and bone resorption. However, more recent studies have shown minimal calcium loss as a result of caffeine consumption, and the amount of calcium lost can be replenished by consuming just one to two tablespoons of milk daily (Heaney, 2002). Additionally, earlier studies that found caffeine decreased bone density may have been due to confounding since participants who already had low calcium intake often consumed a diet low in dietary calcium (Barrett-Connor et al., 1994; Harris and Dawson-Hughes, 1994; Heaney, 2002).

Since vitamin D is a fat soluble vitamin, the majority of it is absorbed with fat in the small intestine via chylomicrons. However, some vitamin D is also transported with amino acids and carbohydrates into the portal system to reach the liver. Studies have shown that in the presence of fat or a large meal, vitamin D (either D_2 or D_3) is absorbed more efficiently. In a small prospective cohort study that consisted of male and female participants who were already taking vitamin D supplements (D_2 or D_3) usually on an empty stomach or with a small meal were not achieving an adequate rise in serum 25(OH)D concentrations. The improvement of absorption was measured by the increase in serum 25(OH)D concentrations. When the vitamin D supplementation regimen was changed to the largest meal (dinner), there was a significant average serum 25(OH)D concentration increase of 58.7% ± 36.7% from baseline. There were no significant differences in vitamin D absorption between those taking the vitamin D_2 form versus the vitamin D_3 form (Mulligan and Licata, 2010). In a one day RCT consisting of three treatment groups (fat free meal group, 30% of calories from fat with a low MUFA (monounsaturated fatty acid): PUFA (polyunsaturated fatty acid) meal group, and 30% of calories from fat with a high MUFA: PUFA meal group, each participant was also provided with a one-time dose of 1250 μg (50,000 IU) of vitamin D_3 at breakfast (Dawson-Hughes et al., 2015). Plasma vitamin D, which is a measure of vitamin D absorption, was measured at baseline and at 10, 12, and 14 hours post supplement consumption. There was a significant increase in absorption in the fat containing group when compared to the no fat group, however no significant changes in absorption in the low MUFA: PUFA group to the high MUFA: PUFA group (Dawson-Hughes et al., 2015). Unlike calcium, no compounds found in foods have been reported to inhibit vitamin D absorption (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D, 2010).

1.5.3 Calcium and vitamin D function/absorption changes during pregnancy and postpartum

From animal studies, calcium passes through the placenta by way of active transport from the mother to the fetus. Calcium binding proteins (CaBP) also are thought to facilitate this process (reviewed by Husain and Mughal, 1992). In rat studies, maternal vitamin D status had no bearing on calcium transport (reviewed by Husain and Mughal, 1992). During a full term pregnancy (37-41 weeks gestation), the fetus obtains approximately 25-30 g of calcium from the mother for its own use which includes the mineralization of the skeleton (Harville et al., 2004; Thomas and Weisman, 2006; reviewed by Kaludjerovic and Vieth, 2010). The highest transport of calcium from mother to fetus occurs in the third trimester where it is estimated that at 35 weeks gestation, the infant utilizes 330 mg of calcium/day in comparison with 50 mg/day occurring at 20 weeks gestation (Thomas and Weisman, 2006). Thus, maternal calcium needs are assumed to be met by with the increase in maternal intestinal calcium absorption from approximately 33-36% before pregnancy to 54-62% by the last trimester (reviewed by Kaludjerovic and Vieth, 2010). Calcium transport decreases in the presence of low maternal plasma calcium as well as decreases uterine blood flow (reviewed by Husain and Mughal, 1992). By 3 months postpartum for both lactating and non-lactating women, calcium absorption returns to its pre-pregnancy state (Olausson et al., 2012). Vitamin D is essential to stimulate calcium absorption in the gut as well as regulate urinary excretion (reviewed by Kaludjerovic and Vieth, 2010). When serum vitamin D concentrations reach a threshold of approximately 80 nmol/L, there is no further increase of calcium absorption (reviewed by Kaludjerovic and Vieth, 2010). Thus, women of child bearing age must consume sufficient dietary calcium to optimize peak bone mass since the fetus and infant’s calcium needs take precedence over maternal needs. With an insufficient amount of calcium in the diet, despite decreased renal excretion and increased intestinal absorption that occurs during pregnancy, this will result in calcium extraction from maternal bones resulting in excessive bone loss (Thomas and Weisman, 2006). Women of child bearing age must consume sufficient calcium to optimize peak bone mass to ensure that they can meet the demands of pregnancy.

The major serum form of vitamin D (25(OH)D) crosses the placenta easily once it is formed at four weeks gestation and concentrations in fetal cord blood will reach 87% of their mother's concentration (reviewed by Kaludjerovic and Vieth, 2010). 25(OH)D gets converted to the active form of calcitriol by both the placenta and fetal kidneys. It is thought that the rise in maternal calcitriol regulates hormones that prevent the induction of inflammatory cytokines that stimulate preterm labor and preeclampsia (reviewed by Kaludjerovic and Vieth, 2010). Therefore, those with lower concentrations of calcitriol would be at greater risk of developing preeclampsia.

1.5.4 Calcium and vitamin D status measures in mother and infant during pregnancy

Unfortunately, there is no "gold standard" measurement of calcium status. Since changes in serum calcium concentrations are more a reflection of metabolic conditions like renal failure or malignancy rather than insufficient intake, serum calcium is not a sensitive biomarker of dietary calcium status (reviewed by Hacker et al., 2012). Dietary calcium intake is the usual way to assess adequacy during pregnancy and can be estimated using a FFQ, 24-hour recall, or food diary. Each of these methods has strengths and limitations that make comparisons between studies difficult. In addition, when trying to use calcium intake as a marker of 'adequacy,' there are other dietary variables that need to be considered including vitamin D intake and status, potential interactions with other nutrients and food components, changes in absorption rate during pregnancy or related status, renal excretion, parathyroid gland functioning, and bone remodeling (reviewed by Kaludjerovic and Vieth, 2010).

Serum 25-hydroxyvitamin D (25(OH)D) concentration is the best indicator of vitamin D status because it accounts for both exogenous and endogenous sources. In the medical community, various professional organizations do not agree on what constitutes a “normal” concentration. The Institute Medicine (IOM) defines a serum 25(OH)D concentration of < 30 nmol/L as deficient, between 30 nmol/L and 50 nmol/L as insufficient, and > 50 nmol/L as normal status for health promotion and disease prevention for both infants and adults. However, according to the Canadian Pediatric Society (CPS), a serum 25(OH)D concentration of < 25 nmol/L is deficient, between 25 nmol/L and 75nmol/L is insufficient, and 75-225 nmol/L is optimal. The differing standards make it problematic for research comparison. From animal studies, concentrations greater than 500 nmol/L (10 times the normal limit) were potentially toxic with more serious metabolic adverse effects such as hypercalcemia and hyperphosphatemia (reviewed by Kaludjerovic and Vieth, 2010).

The IOM standard applies to the general population, however due to the limited research in this area during pregnancy and lactation, it is uncertain what an acceptable vitamin D concentration should be during these periods. Using the current standard, there is emerging evidence that 10 µg (400 IU)/day may not be enough to achieve normal serum vitamin D concentrations during pregnancy and lactation which can be preventative against rickets and other childhood diseases of the offspring as well as disease development later in life. In fact, one study reported that 10% of Caucasians and 46% of black neonates have serum 25(OH)D concentrations in the insufficient range (Bodnar et al., 2007b) despite maternal multivitamin use and a healthy diet. The CPS has recommended that pregnant women increase their intake to 50 μg/day (2000 IU)/day based on several studies that show no adverse effects with this type of consumption (reviewed by Godel, 2007). A small randomized study by Hollis et al. (2004) that examined the efficacy of maternal vitamin D supplementation on maintaining normal concentrations of serum 25(OH)D of mothers and their exclusively breast fed infants found significant increases in serum 25(OH)D concentrations with greater supplementation than the RDA. The first group received 40 μg (1600 IU)/day of D_2 and 10 μg (400 IU) of D_3, and the second group was given 90 μg (3600 IU)/day of D_2 and 10 μg (400) IU of D_3 for a total of three months. The total circulating 25(OH)D concentrations from the first group increased from 68.9 ± 8.2 to 90.1 ± 5.7 nmol/L ($p < 0.05$) and total circulating 25(OH)D concentrations from the second group increased from 82.1 ± 6 to 111 ± 9.7 nmol/L ($p< 0.04$). No adverse effect were observed with any of the participants and serum calcium concentrations all remained within the normal limit. The breast milk of women in the 100 μg (4000 IU) group had greater serum 25 (OH)D concentrations than those supplemented 50 μg (2000 IU) (35.5 ± 3.5 to 69.7 ± 3.0 IU/L and 40.4 ± 3.7 to 134.6 ± 48.3 IU/L) which suggests they would better meet vitamin D requirement for their infants. Two other clinical trials support the safety of consuming greater amounts of vitamin D. Vieth et al. (1986) studied healthy men and women with doses of vitamin D up to 100 μg (4000 IU)/day for a duration of two to six months. Again, no adverse effects were observed. According to Vieth et al. (2004), both groups (15 μg (600 IU)) and (100 μg (4000 IU)) had significantly higher vitamin D status from baseline and higher concentrations were observed with greater supplementation at two to six months post supplementation. A double-blinded placebo controlled intervention trial in Ireland identified that a daily dose of 19.9 μg (796 IU), 28 μg (1120 IU), and 41.1 μg (1,644 IU) of supplemental vitamin D_3 was need to achieve a serum 25(OH)D concentration above 25.0 nmol/L (deficient), 37.5 nmol/L (insufficient), 50.0 nmol/L (insufficient), and 80.0 nmol/L (normal according to the IOM), respectively. From fall to winter, vitamin D concentration fell for all four groups receiving supplemental vitamin D_3 (placebo- 0 IU, 5 μg (200 IU), 10 μg (400 IU), and 15 μg (600 IU)) (Cashman et al., 2008). This data may substantiate the need to change the RDA and UL especially in the fall and winter months in northern countries in order to obtain a serum 25(OH)D concentrations within the 'normal' range. The results of earlier studies have not been replicated and the subjects were male which were extrapolated to women, particularly women during pregnancy and postpartum.

In conclusion, from observational studies, there is a reported high prevalence of vitamin D deficiency as well as insufficiency in expectant mothers (Ward et al., 2007; Bodnar et al., 2007b; Ginde et al., 2010; reviewed by Hyppönen, 2011). There is also a direct relationship between maternal and fetal cord vitamin D concentration as well as infant vitamin D status (Ward et al., 2007; Viljakainen et al., 2010; reviewed by Hyppönen, 2011; Thiele et al., 2013).

1.6 Estimating calcium and vitamin D consumption during pregnancy and postpartum (diet, supplement, and total): a critical review of the literature

1.6.1 Dietary Reference Standards

Table 1.4 summarizes the key studies that have estimated dietary calcium and vitamin D intake during pregnancy and postpartum. It should be noted that not all developed countries use the same standards to determine intake adequacy. In the United Kingdom (UK), the recommended intake of calcium during pregnancy and lactation is 700 mg/day and 1250 mg/day, respectively. The Nordic Nutrition Recommendations (NNR) for calcium is 900 mg/day. Both the UK and the Nordic countries recommend 10 μg (400 IU)/day of vitamin D during pregnancy and lactation. In Canada and the United States, the EAR for calcium and vitamin D are 800 mg/day and 10 µg (400 IU)/day, respectively, and the RDA for calcium and vitamin D are 1000 mg/day and 15 µg (600 IU)/day, respectively.

1.6.2 Calcium intake during pregnancy and postpartum

In developed countries, regardless of what standard used, women were generally reported to be meeting dietary guidelines for calcium throughout pregnancy with diet alone which was true for women of childbearing age in Canada as well. However, women of childbearing age in Canada had lower intakes from diet than pregnant and postpartum women. According to Statistics Canada Canadian Community Health Survey (CCHS) from 2004, women ages 19-30 and 31-50 consumed 864 ± 26 mg/day and 828 ± 18 mg/day, respectively. A study on calcium intake in early (mean: 13.8 weeks gestation) and late (mean: 36.6 weeks gestation) pregnancy by Harville et al. (2004), found that the 385 Caucasian and African American participants had a mean and median dietary intake of 1463 mg/day and 1243 mg/day, respectively, which met the EAR. The mean and median total intakes increased to 1671 mg/day and 1482 mg/day during pregnancy, respectively, when accounting for supplement use. Estimated total calcium intake in this study was higher than other studies which may be attributed to several participants enrolled in Women, Infants, and Children (WIC), which provides expectant low-income women nutrition counseling as well as funds to purchase calcium rich food items such as milk and cheese. If fortified foods were not included in the FFQ, one can predict that calcium intake would be even higher. The Block Questionnaire, which was the FFQ used, has limited reliability and validity since it has a history of overestimating calcium intake (Block et al., 1992; Harville et al. 2004). Harville et al. (2004) combined dietary intake collection from early pregnancy (range: 8-18 weeks gestation) and late pregnancy (range: 31-46 weeks gestation) which may be problematic given the differences in dietary intake between the two time points related to nausea and altered taste perception which are common symptoms of morning sickness in early pregnancy and often lead to altered food/beverage and caloric consumption that improves as pregnancy progresses (Rifas-Shiman et al., 2006). Another significant limitation to this study was that vitamin D intake and status were not measured. The Norwegian Mother and Child Cohort Study (MoBa) by Haugen et al. (2008), assessed the contribution of supplements to the total nutrient intake of various micronutrients, including calcium. Dietary and supplement intake was collected at 17-24 weeks gestation using a semi-quantitative FFQ, which asked about intake since becoming pregnant. Like Harville et al. (2004), estimated calcium intake was obtained predominately from diet. There were no significant differences in intake when comparing supplement users (SU) who consumed an average of 1053 ± 426 mg/day to non-supplement users (NSU) consuming 1007 ± 435 mg/day from diet. The estimated mean and median intake from diet alone was above the EAR. Even though calcium supplementation was not substantial to overall intake, the most common calcium containing supplements were the following: 30.8% supplemented with a multivitamin with minerals, 16.3% with a multivitamin, and 3.2% from calcium tablets.

When reviewing the literature, it appears that North American women use a multivitamin to meet micronutrient needs more frequently than European women. For example, in an observational study published by the *Irish Medical Journal*, of the 450 women participating, only 10% of women reported consuming a multivitamin and mineral supplement during pregnancy (Tarrant et al., 2011). In North America, even though the majority of pregnant women's daily reported calcium intake comes from the foods and beverages in their diet, the contribution from supplements is not negligible. Rodríguez-Bernal et al. (2010), which looked at diet quality in early pregnancy, reported that 52.1% of the women took a calcium containing supplement and Sullivan et al. (2009) found that 78% of participants took a multivitamin throughout pregnancy. Supplement intake was found to be significantly higher for pregnant women than non-pregnant women (Sullivan et al., 2009). Project Viva, by Rifas-Shiman et al. (2006), studied 1543 pregnant women to assess the changes in intake of selected foods and food groups from the first and second trimester using a validated FFQ used in the Nurse's Health Study and a separate supplement questionnaire. Both first and second trimester mean calcium estimated intake from diet (food and beverages) was 1118 ± 347 mg and 1168 ± 344 mg/day, respectively, which was above the EAR. Estimate mean total calcium intake (diet + supplements) from the first and second trimesters was 1320 ± 418 mg/day and 1435 ± 387 mg/day, respectively.

Derbyshire et al. (2009) from the UK was the only study reviewed that estimated dietary intake during each trimester (13, 25, and 35 weeks gestation) as well as 6 weeks postpartum. Since the aim of the study was to quantify micronutrient intake from diet from gestation through the postpartum period, supplement intake was not assessed. Interestingly, there were no significant differences found in estimated calcium intake between pregnancy and postpartum. The mean estimated calcium intake from diet was 913 ± 332 mg, 883 ± 265 mg, 945 ± 283 mg, and 882 ± 277 mg/day for each trimester and postpartum. Less than 50% of women met the UK calcium recommendation during pregnancy, and only 17% met the recommendation during the postpartum period. The estimated mean calcium intake fell short of the Canadian guidelines as well even though participants were of high socioeconomic status, older (33.2 ± 4.55 years old), and had normal pre-pregnancy body mass index (BMI) status (24.7 kg m^2) which are predictors of better nutritional intake (Derbyshire et al., 2009). Thus, a socioeconomic status difference could not explain the dissimilarity from previous studies that reported mean/median intakes during pregnancy that met the DRI (Harville et al., 2004). However, the women in this study were also not meeting recommendations for other vitamins and minerals such as folate, iron, selenium, and potassium which are nutrients this population are not at risk for deficiency for. This suggests that overall poor nutrient density in the diet may have contributed to lower calcium intake. A Canadian study by Mannion et al. (2007) of exclusively breast feeding women (n= 175) found that milk consumers (defined as > 250 mL/day) consumed an average of 1379 ± 471 mg/day of calcium meeting the EAR which was significantly higher than that of women that restricted milk (895 ± 330 mg/day of calcium, meeting the EAR but falling short of the RDA). Non-milk drinkers were only able to meet the RDA with an intake of 1287 ± 470 mg/day when supplementation was considered compared to milk drinkers that consumed 1564 ± 487 mg/day. Mannion et al. (2007)'s findings regarding calcium intakes differs from other studies because milk drinking status was identified as a co-variable. Other studies did not control for this variable which is known to be associated with higher calcium intakes. It is therefore possible that the differences between studies may have been due to the frequency of milk consumption by the participants.

1.6.3 Vitamin D intake during pregnancy and postpartum

Regardless of what standard used, pregnant women are consistently reported to not meet the EAR for vitamin D through their diet which is consistent with women of childbearing age as well. According to Statistics Canada CCHS from 2004, 91-95% of women of childbearing age were not meeting the EAR with diet alone, and even with the addition of supplements, 71-81% were still not meeting the EAR. Pregnant women heavily rely on supplement usage to reach the current recommendations. Based on the literature review, diet contributed to less than 50% of total vitamin D intake in pregnant and postpartum women (Rifas-Shiman et al., 2006; Mannion et al., 2007; Haugen et al. 2008). In a large Norwegian study that looked at the nutrient intake of

SU versus NSU in pregnancy, no significant differences were found in estimated dietary intake (3.5 µg (140 IU) ± 2.5 µg (100 IU) vs. 3.5 µg (140 IU) ± 2.7 µg (108 IU)/day). The average intake by SU was 10.1 µg (404 IU) ± 9.3 µg (388 IU)/day which resulted in a total estimated intake of 13.6 µg (544 IU) ± 9.7 µg (388 IU/day). Only 37% of the total cohort, 65% of vitamin D SU, and 1% of NSU met the met the NNR (10 µg (400 IU)/day). The limitations of the study included low participation rate (41.6%) of women recruited and difficulty obtaining accurate information on the nutrient content of supplements (Haugen et al., 2008). A UK study also supported that women have significantly lower vitamin D consumption than the recommendations during each trimester of pregnancy (Derbyshire et al., 2009). In this study, mean estimated dietary intake from a four to seven day weighted food dairy during each trimester was 1.86 µg (74 IU) ± 0.93 µg (37.2 IU), 2.59 µg (103.6 IU) ± 1.74 µg (69.6 IU), and 2.18 µg (87.2 IU) ± 1.31 µg (52.4 IU)/day, which was well below both the UK and Canadian recommendations (Derbyshire et al., 2009). Project Viva found the mean estimated dietary intake in the first and second trimester was 4.5 µg (216 IU) ± 2.93 µg (117 IU)/day and 5.75 µg (230 IU) ± 2.9 µg (116 IU)/day, respectively, and the total estimated intake from diet and supplements was 12.6 µg (504 IU) ± 5.25 µg (210 IU) and 15.05 µg (602 IU) ± 4.65 µg (186 IU)/day, respectively (Rifas-Shiman et al., 2006). This was the only study reviewed where the EAR and RDA were met by the women in the study through diet and supplements. This study only estimated intake during the second trimester and it is not known what the intake was in late pregnancy and postpartum. While the higher socioeconomic status of participants posed as a bias to this study as women who have greater education had a higher intake of vitamin D, the majority of participants from the APrON (Alberta Pregnancy Outcomes and Nutrition) study are of high socioeconomic status (Harville et al., 2004; Derbyshire et al., 2009). There is currently a lack of research in estimating intake across pregnancy and through the postpartum period as most studies have selected one or two time points. A Finnish study by Viljakainen et al. (2010) of Caucasian women's vitamin D status and its influence on bone variables of infants, looked at calcium and vitamin D intake from diet and supplements. Intake was estimated using a semi-quantitative FFQ at approximately 35 weeks gestation. Researchers did not specify whether or not the FFQ was retrospective from the first trimester or contained questions regarding fortified foods and beverages. From the 124 participants, the estimated mean dietary and total vitamin D intake was 7.8 µg (312 IU) ± 3.3 µg (132 IU) /day and 14.3 µg (572 IU) ± 5.8 µg (232 IU)/day, respectively. Although calcium intake was estimated, it was not reported in this manuscript. As other previous research showed, recommendations were not met with diet but were met with total intake.

Pregnant women consume greater vitamin D from diet and supplements than their non-pregnant counterparts. According to Bailey et al. (2010) which reviewed NHANES 2005-2006 from the United States, women ages 19-30 consumed an estimated average of 3.6 µg (144 IU) ± 0.3 µg (12 IU)/day from diet and about the same amount from supplements resulting in a total estimated intake of 5.8 µg (232 IU) ± 0.3 µg (12 IU)/day. Older women ages 31-50 reported consuming more vitamin D from diet which was 4.4 µg (176 IU) ± 0.3 µg (12 IU)/day as well as supplements making total estimated intake 7.7 µg (308 IU) ± 0.5µg (20 IU)/day.

During the postpartum period, estimated vitamin D intake does not appear to be higher than during pregnancy. In a study from the UK of ethnically and racially diverse postpartum women of low birthweight babies, estimated mean vitamin D intake by Caucasian women was 2.4 µg (96 IU)/day and a higher estimated dietary intake was found among Africans (4.72 µg (188 IU)), Caribbean-Africans (3.18 µg (127.2 IU)), and Asians (2.7 µg (108 IU))/day. Despite non-Caucasian women having significantly greater estimated dietary intake, all groups were still below the Canadian EAR (Rees et al., 2005). Most women in the study were not breastfeeding. A second UK study of Caucasian women also found an estimated dietary vitamin D intake of 2.31 µg (92 IU) ± 1.75 µg (70 IU)/day during the postpartum period which was well below the Canadian recommendations (Derbyshire et al., 2009). A Canadian study by Mannion et al. (2007), found that milk drinkers had significantly greater estimated mean intake (6.3 µg (252 IU) ± 4 µg (160 IU)/day) compared to non-milk drinkers (2.2 µg (88 IU) ± 2 µday g (80 IU)/day) of vitamin D in their diet. With supplements, milk consumers had an estimated total of 10.7 µg (428 IU) ± 4.9 µg (196 IU/day) of vitamin D which was twice that of non-milk drinkers (6.1 µg (244 IU) ± 4.7 µg (188 IU)/day). Only milk consumers with supplements met the EAR for vitamin D. The limitations of this study which may have altered results included difficulty recruiting and retaining participants, exclusion of non-English speakers, and the timing of dietary collection (outcome was low birth weight which is more a reflection of nutrition during pregnancy however dietary intake was measured 8-12 weeks postpartum). When comparing milk consumption of pregnant women to Canadian women of childbearing ages (17-30 and 31-50), only 25% and 28%, respectively, were consuming the recommended two servings of milk products per day recommended by Canada’s Food Guide making it difficult to meet vitamin D recommendations unless other dairy products were consumed in their place (Statistics Canada, CCHS, 2004).

1.6.4 Calcium and vitamin D supplement usage during pregnancy and postpartum

Throughout pregnancy in developed countries, 50%-89% of women reported consuming a multivitamin (Harville et al., 2004; Mannion et al., 2007; Haugen et al., 2008; Rodríguez-Bernal et al., 2010; Viljakainen et al., 2010) which was higher than 47 % of non-pregnant women of childbearing age (Sullivan et al., 2009). Predictors of multivitamin use in pregnant women included income and marriage status (Rodríguez-Bernal et al., 2010). Despite consuming supplements, they only provided 12%-18% of total calcium intake during pregnancy (Harville et al., 2004; Rifas-Shiman et al., 2006; Haugen et al., 2008). Although, only 3.2% of pregnant women reported using a calcium single supplement (Haugen et al., 2008), calcium intake from antacids to treat gastric reflux was common; nearly 25% of participants consumed 10% of calcium needs from a calcium based antacid (Harville et al., 2004; Thomas and Weisman, 2006). Based on the average calcium supplemented during pregnancy, one can conclude the majority of calcium supplemented came from a multivitamin and less from a single supplement. According to a study of calcium and vitamin D intake from NHANES 2003-2006 from the United States, vitamin D intake from supplements for 19-30 year old women was among the lowest of the eight other identified age brackets which was 7.5 µg (300 IU) ± 0.7 µg (28 IU)/day and only 21 ± 4% of participants were users (Bailey et al., 2010). As opposed to this, in a Finnish study, 80% of pregnant women reported using a vitamin D containing supplement (Viljakainen et al., 2010). Based on the literature review, vitamin D from supplements appears to contribute 40-65% of estimated total vitamin D intake (Rifas-Shiman et al., 2006; Mannion et al., 2007; Haugen et al., 2008). Unlike calcium, the majority of vitamin D in the diet comes from a supplement.

Table 1.4: Key findings of calcium and vitamin D intake studies

Study/type/ title **Sample size**	**Nutrient(s) studied** **Dietary intake method used**	**Estimated dietary intake (mean unless otherwise specified) & trimester collected** mg/day & µg (IU)/day	**Estimated supplement intake (mean unless otherwise specified) & trimester collected** mg/day & µg (IU)/day	**Estimated total intake (mean unless otherwise specified) & trimester collected** mg/day & µg (IU)/day	**Variables influencing intake**

● Harville et al., 2004 (USA) ● longitudinal study ● Calcium intake during pregnancy among white and African-American pregnant women in the United States ● n= 385	● Ca ● FFQ	● early & late pregnancy- 1463 mg ● median early & late pregnancy- 1243 mg	● median early pregnancy- 200 mg ● median late pregnancy- 500 mg	● early & late pregnancy- 1671 mg ● median early & late pregnancy- 1482 mg	● total intake- age, smoking status, race, parity, weight gain during pregnancy, education level, prenatal vitamin/antacid use, income, milk drinking status, lactose intolerance, and income
● Rifas-Shiman et al., 2006 (USA) ● prospective cohort study ● Changes in dietary intake from the first to the second trimester of pregnancy ● n= 1543	● Ca & Vit D ● semi-quantitative FFQ	● Ca (1st)- 1118 ± 347 mg ● Ca (2nd)- 1168 ± 344 mg ● Vit D (1st)- 5.4 µg (216 IU) ± 2.93 µg (117 IU) ● Vit D (2nd)- 5.75 µg (230 IU) ± 2.9 µg (116 IU)	● not reported	● Ca (1st)- 1320 ± 418 mg ● Ca (2nd)- 1435 ± 387 mg ● Vit D (1st)- 12.6 µg (504 IU) ± 5.25 µg (210 IU) ● Vit D (2nd)- 15.1 µg (602 IU) ± 4.65 µg (186 IU)	● age, marital status, supplement use, education level, race, parity, nausea/ vomiting, cravings/ aversions, and BMI
● Derbyshire et al., 2009 (UK) ● Habitual micronutrient intake during and after pregnancy in Caucasian Londoners ● n= 42	● Ca & Vit D ● 4 or 7 day weighted food diary	● Ca (1st)- 913 ± 332 mg ● Ca (2nd)- 883 ± 265 mg ● Ca (3rd)- 945 ± 283 mg ● Ca (PP)- 882 ± 277 mg ● Vit D (1st)- 1.86 µg (74 IU) ± 0.93 µg (37.2 IU) ● Vit D (2nd)- 2.59 µg (104 IU) ± 1.74 µg (69.6 IU) ● Vit D (3rd)- 2.18 µg (87 IU) ± 1.31 µg (52.4 IU)	● n/a	● n/a	● age, smoking status, race, BMI, and socioeconomic status

		● Vit D (PP)- 2.31 µg (92 IU) ± 1.75 µg (70 IU)			
● Haugen et al., 2008 (Norway) ● retrospective cohort study ● Dietary supplements contribute substantially to the total nutrient intake in pregnant Norwegian women ● n= 40,108	● Ca & Vit D ● semi-quantitative FFQ	● Ca suppl. user (SU)- 1053 ± 426 mg ● Ca median (SU)- 982 mg (497,1843) ● Ca non-suppl. user (NSU)- 1007 ± 435 mg ● Ca median (NSU)- 927 mg (443,1854) ● Vit D (SU)- 3.5 µg (140 IU) ± 2.5 µg (100 IU) ● Vit D median (SU)- 3.1 µg (124 IU) (1.0,7.1) ● Vit D (NSU)- 3.5 µg (140 IU) ± 2.7 µg (108 IU) ● Vit D median (NSU)- 3 µg (120 IU) (0.9,7.1) ● data collection: 17-24 wks. gestation (intake since becoming pregnant)	● Ca (SU)- 290 ± 290 mg ● Ca median (SU)- 200 mg (4,1000) ● Vit D (SU)- 10.1 µg (404 IU) ± 9.3 µg (372 IU) ● Vit D median (SU)- 6.8 µg (272 IU) (1.1, 29) ● data collection: 17-24 wks. gestation	● Ca (SU)- 1270 ± 483 mg ● Ca median (SU)- 1192 mg (631,2143) ● Vit D (SU)- 13.6 µg (544 IU) ± 9.7 µg (388 IU) ● Vit D median (SU)- 10.4 µg (416 IU) (3.5,32.6) ●Ca (total)- 1076 ± 444 mg ● Vit D (total)- 10.7 µg (428 IU) ± 9.5 µg (360 IU) ● data collection: 17-24 wks. Gestation	● supplement use, smoking status, parity, age, education, and BMI
● Viljakainen et al., 2010 (Finland) ● cross-sectional study ● Maternal vitamin D status determines bone variables in the newborn ● n= 125	● Vit D ● semi-quantitative FFQ	● 7.8 µg (312 IU) ± 3.3 µg (132 IU) ● data collection: 3rd trim.	● 6.6 µg (264 IU) ± 4.8 µg (192 IU) ● data collection: 3rd trim.	● 14.3 µg (572 IU) ± 5.8 µg (232 IU) ● data collection: 3rd trim.	● supplement use- study mostly about nutritional status vs. intake

● Mannion et al., 2007 (Canada) ● cohort study ● Lactating women restricting milk are low on select nutrients ● n= 175	● Ca & Vit D ● 3-4 day dietary recall	● Ca milk non-restrictor (NRS)- 1379 mg ± 471 mg ● Ca milk restrictor (RS)- 895 mg ± 330 mg ● Ca median (NRS)- 1326 mg ●Ca median (RS)- 895 mg ● Vit D (NRS)- 6.3 µg (252 IU) ± 4 µg (160 IU) ● Vit D (RS)- 2.2 µg (88 IU) ± 2 µg (80 IU) ● Vit D median (NRS)- 5.65 µg (226 IU) ● Vit D median (RS)- 1.82 µg (73 IU) ● data collection: 2, 4, and 6 mos. PP	● Ca milk non-restrictor (NRS)- 205 mg ● Ca milk restrictor (RS)- 392 mg ● Vit D (NRS)- 4.4 µg (176 IU) ●Vit D (RS)- 3.9 µg (156 IU) ● data collection: 2, 4, and 6 mos. PP	● Ca (NRS)- 1564 ± 487 mg ● Ca (RS)- 1287 ± 470 mg ● Vit D (NRS)- 10.7 µg (428 IU) ± 4.9 µg (196 IU) ● Vit D (RS)- 6.1 µg (244 IU) ± 4.7 µg (188 IU) ● data collection: 2, 4, and 6 mos. PP	● milk restriction secondary to perceived infant intolerances (colicky behavior, mucus production, GI upset) or own lactose intolerance, caloric intake, and supplement intake
● Sullivan et al., 2009 (USA) ● Cross-sectional study ● Multivitamin use in pregnant and non-pregnant women: results from the Behavioral Risk Factor Surveillance Survey ● all women n= 19,341 ● pregnant women	● No nutrients in particular-general supplement usage	● n/a- not quantified	● 78% of pregnant women used multivitamin	● n/a- not quantified	● age, higher income*, physical activity, race/ethnicity, marital status*, healthcare coverage status, health condition, smoking status, and BMI * indicates reaching statistical significance for pregnant women

● n= 788					
● Rodríguez-Bernal et al., 2010 (Spain) ●Cohort study ● Diet quality in early pregnancy and its effects on fetal growth outcomes: the Infancia y Medio Ambiente (Childhood and Environment) Mother and Child Cohort Study in Spain ● n= 787	● diet quality studied (Alternate Health Eating Index or AHEI) for Ca ● FFQ-intake from last menstrual period to 1st trim.	● n/a- not reported	● 52.1 % of pregnant women used Ca containing suppl.	● n/a- not reported	● age*, smoking, education income, and educational status * indicates reaching statistical significance
● Rees et al., 2005 (UK) ● retrospective study ● The nutrient intakes of mothers of low birth weight babies – a comparison of ethnic groups in East London, UK ● n= 165	● Ca & Vit D ● 7-day food record	● Caucasian Ca (PP)- 780 mg ● African Ca (PP)- 565 mg ● Asian Ca (PP)- 629 mg ● Afro-Caribbean Ca (PP)- 658 mg ● Caucasian Vit D (PP)- 2.4 µg (96 IU) ● African Vit D (PP)- 4.72 µg (189 IU) ● Asian Vit D (PP)- 2.47 µg (99 IU) ● Afro-Caribbean Vit D (PP)- 3.18 µg (127 IU)	● n/a- women taking supplements were excluded from study	● (see dietary intake)	● socioeconomic status, smoking, ethnicity*, caloric intake, and dairy consumption
● Oken et al., 2007 (USA) ● Diet during pregnancy and	● Ca & Vit D ● SFFQ	● n/a- not reported	● n/a- not reported	● Ca- 1309 ± 416 mg	● parity, age, marriage status, education, BMI, and ethnicity

risk of preeclampsia or gestational hypertension ● prospective cohort study ● n= 1718				● Vit D- 12.4 µg (496 IU) ± 5.25 µg (210 IU) ● data collection: 1st trim.	

1.6.5 Populations at the greatest risk for not meeting recommendations for both calcium and vitamin D during pregnancy and postpartum

From the review of the literature, the following groups of women were at greatest risk of low dietary and/or supplemental calcium intake during pregnancy and/or the postpartum period: non-Caucasians, multiparous, young age, high BMI, low caloric intake, poor diet quality (micro and macronutrients alike), less education, smokers, low socioeconomic status, those not taking dietary supplements, and those that restricted milk consumption (Harville et al., 2004; Rifas-Shiman et al., 2006; Mannion et al., 2007; Derbyshire et al., 2009). A Canadian study by Mannion et al. (2007) of exclusively breast feeding women found that milk consumers (> 250 mL/day) had significantly higher ($p< 0.05$) dietary calcium intake than those that restricted milk consumption. 'Milk restrictors' median estimated calcium intake was only slightly above the EAR for calcium and did not meet the recommendations for protein and other micronutrients. When supplements were used by 'milk restrictors', they were more likely to meet recommendations (Mannion et al., 2007). In those defined as 'milk drinkers' in this study, 48% of total daily calcium came from dairy products.

In summary, few studies have looked at both vitamin D and calcium intake during pregnancy and postpartum. Since a large proportion of daily vitamin D comes from supplements rather than dietary sources in pregnant women, those that did not take vitamin D containing supplements were at greater risk for lower intake. Other characteristics of women at risk included low caloric intake, younger age, higher BMI (classified as overweight or obese), less education, being a smoker, and being multiparous (Rifas-Shiman et al., 2006; Haugen et al., 2008).

1.6.6 Dietary patterns of intake observed in pregnancy and postpartum

Dietary patterns have been measured during pregnancy, however few studies have looked at intake patterns during the postpartum period. Harville et al. (2004) reported that the highest proportion of calcium was derived from milk, then cheese, bread, and mixed dishes during pregnancy. Women who consumed less than 600 mg/day had a smaller proportion of calcium from milk and more from cheese, bread, and mixed dishes which corroborates with Mannion et al. (2007)'s results that women who drank milk had higher intakes. In this study, women who reported milk intake during pregnancy of < 250 mL/day still consumed 48% of calcium from dairy products which included milk, yogurt, and cheese while other sources included grain products (12%), fruits/vegetables (11%), and mixed dishes and other foods for the remaining proportion of intake. Rifas-Shiman et al. (2006) found a 22% mean intake increase of skim and 1% dairy and a 15% increase in whole dairy from the first to the second trimester. However, when looking at relative changes in quartiles in food and food group consumption, 50% of the women stayed in the same quartile (37% increased or decreased one quartile, 10% increased or decreased two quartiles, and few went from highest to lowest and vice versa). This showed that food intake changed significantly for half the women in the first and second trimester while the other half stayed the same. There were few studies that looked at foods and beverages that contributed to dietary vitamin D intake across gestation which was likely due to the majority of intake coming from supplements. Rifas-Shiman et al. (2006) identified how many servings pregnant women were consuming of various food categories, however the groups were not well defined making it difficult to assess the vitamin D content of a food group. For example, categories such as “fish” or “skim or 1% dairy foods” were used, however not all fish are high in vitamin D and not all dairy products are fortified like cow’s milk.

1.6.7 Vitamin D status- influences and importance

Biologically, those with darker pigmentation and increased age do not synthesize vitamin D in the skin well which puts them at greater risk of deficiency (reviewed by Kaludjerovic and Vieth, 2010; Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, 2012). A study that compared the serum 25(OH)D concentrations of young African-American and Caucasian women measures at four time points (February-March, June or July, October or November, and the following February-March) found that at all time points, African-American women had lower serum 25(OH)D concentrations even when controlling for confounding variables. In the winter when synthesis decreases, the mean serum 25(OH)D concentration was 30.2 ± 19.7 nmol/L in African-Americans and 60.0 ± 21.4 nmol/L in Caucasian women (Harris and Dawson-Hughes, 1998). The RDI accounts for minimal vitamin D synthesis from sunlight but may be more difficult to obtain in the winter months especially in northern areas of the world. The CPS and Hyppönen (2011) reported that in Edmonton, Alberta which is located at 52° north latitude, vitamin D_3 skin production is almost non-existent from October to April. Since women are not meeting the EAR for vitamin D with diet and supplements, there has been a debate whether or not the recommendations should change during the darker months in the Northern Hemisphere when stores deplete and synthesis from skin decreases or is absent (reviewed by Hyppönen, 2011).

Increased adipose mass (obesity) is associated with lower 25(OH)D concentrations (Ross et al., 2011). It has been reported that obese individuals require a larger amount of vitamin D than their normal bodyweight counterparts to maintain the same serum 25(OH)D concentrations (Ross et al., 2011). It is believed that vitamin D is stored in greater quantities in adipose tissue than muscle tissue; however the vitamin D in adipose tissue is not as readily available to release vitamin D when needed (Ross et al., 2011). With moderate weight loss while not changing dietary intake or sun exposure, studies have shown that 25(OH)D concentrations increase (Ross et al., 2011).

Limited research has been conducted on pre-pregnancy BMI and its impact on maternal status during pregnancy as well as newborn vitamin D status at birth. Two studies showed that obese women had significantly lower serum 25(OH)D concentrations than their normal BMI counterparts during pregnancy (early and late) even after controlling for confounding variables such as race, ethnicity, season, age, multivitamin use prior to conception, and physical activity (Bodnar et al., 2007a; Tomedi et al., 2013). Obese women were also more likely to have vitamin D deficiency (defined as < 50 nmol/L). When compared to a woman with a normal BMI of 22, a woman with an overweight BMI of 28 and an obese BMI of 34 was 1.4 (95% CI: 1.1-1.9, $p < 0.01$) and 2.1 (95% CI: 1.2-3.6, $p < 0.01$) times more likely to be vitamin D deficient during pregnancy (Bodnar et al., 2007a). Neonates of obese women also had significantly lower cord concentrations of serum 25(OH)D than their normal BMI counterparts (Bodnar et al., 2007a). When compared to a woman with a normal BMI of 22, a woman with a BMI of 28 (overweight) and BMI of 34 (obese) was 1.5 (95% CI: 1.1-1.9, $p < 0.05$) and 2.1 (95% CI: 1.2-3.8, $p < 0.05$) times more likely give birth to a vitamin D deficient infant (Bodnar et al., 2007a). Therefore, pregnant and lactating women who already have poor dietary intake of vitamin D, have limited sun exposure due to seasonality, are overweight or obese, wear modest clothing for cultural or religious reasons, use sunscreen, live in locations with poor air quality, and live at a northern latitude which decreases sun exposure, are at greatest risk for vitamin D insufficiency and deficiency status.

There has been no research to this author's knowledge on the impact of changes in body fat composition on vitamin D status during pregnancy. This has been in part due to the limited methods that are safe as well as valid to use in pregnant populations (Widen and Gallagher, 2014). Gestational weight gain in association with vitamin D status, has been researched in a small number of studies. In a prospective mother-offspring birth cohort study in the UK, greater weight gain was associated with a decrease in serum 25(OH)D concentrations from early to late pregnancy. Mean gestational weight gain was 10.7 ± 4.3 kg and median BMI was 24.2 (interquartile range:21.9-27.4) which was either below or meeting gestational weight gain guidelines for most participants (Moon et al., 2015). However, since hemodilution occurs with expanding blood volume during pregnancy, one cannot discern whether this may be the underlying reason for lower serum 25(OH)D concentration. Additionally, there may be difficulty in mobilizing vitamin D stores from adipose tissue as observed from obesity studies as previously discussed (Ross et al., 2011; Moon et al., 2015).

1.6.8 Calcium and vitamin D in breast milk and formula

Infants are estimated to absorb about 55-60% of calcium intake (Ross et al., 2011). In formula fed infants, fractional calcium absorption is only 40%, however, the calcium content of formula is doubled that of human breast milk to account for the decrease in absorption **(**Ross et al., 2011). From thirty days to six months, the average calcium content of breast milk is 250-400 mg/L according to a United States and European study (Kalkwarf, 1999; Olausson et al., 2012)**.** The AI for an infant age 0-6 months is 200 mg/day.

There is conflicting evidence as to whether maternal calcium intake can influence the calcium content of breast milk. According to Ortega et al. (1998), women who consume higher amounts of calcium during pregnancy may have a higher calcium content in breast milk. Women that had a calcium intake of < 1100 mg/day had a calcium concentration of 5.95 ± 1.56 mmol/L in breast milk which was significantly less than those with intake > 1100 mg/day (6.82 ± 1.31 mmol/L). However, RCTs have not been able to confirm this observation. A RCT of Gambian pregnant women in Africa, who are known to have low dietary calcium intake (300-400 mg/day), showed that when given 1500 mg of calcium as (3) 500 mg/day doses as compared to those on a placebo, it had no significant effect on infant outcomes like gestational age, birth weight, and bone mineral growth. These women were also followed during the postpartum period and the calcium content of breast milk was analyzed. There were no significant differences in the calcium content of the breast milk between the two groups supporting the physiological hypothesis that maternal calcium stores in skeletal bone are used to support the calcium content in breast milk in the presence of insufficient intake (Jarjou et al., 2006). In support of this, Kalkwarf (1999) found that maternal bone mass during breast feeding decreased by approximately 5-10% during the peak breast feeding period (two to six months), however bone mass increased back to pre-pregnancy mass at six to twelve months post weaning, suggesting physiological mechanisms to replenish bone calcium content.

Vitamin D_3 is the preferred form of supplementation and may be more effective at raising 25(OH)D concentrations because it is thought to have improved binding capabilities than vitamin D_2 to vitamin D binding receptors and greater affinity for vitamin D binding proteins that are responsible for delivering vitamin D to the adipose and muscle tissue as well the kidney and liver for bio-activation (Hollis, 1984). In a meta-analysis by Tripkovic et al. (2012) that reviewed the efficacy of vitamin D_2 compared to vitamin D_3 supplementation in raising serum 25(OH)D concentration, supported that vitamin D_3 supplementation was more effective in improving vitamin D status. Of the ten randomized controlled trials analyzed, eight showed a greater increase in vitamin D status when vitamin D_3 was given compared to vitamin D_2 at the same dosage (Tjellesen et al., 1986; Trang et al., 1998; Armas et al., 2004; Romagnoli et al., 2008; Glendenning et al., 2009; Leventis and Kiely, 2009; Binkley et al., 2011; Heaney et al., 2011). In a double-blinded RCT by Trang et al., (1998), participants taking 100 μg (4000 IU)/day of vitamin D_3 for two weeks experienced a 1.7 times greater increase in serum 25(OH)D concentrations than those in the vitamin D_2 group receiving the same dosage. In a single-blinded RCT by Heaney et al. (2011), participants given 1250 μg (50,000 IU)/day of vitamin D_3 for twelve weeks had an 87% greater increase in serum 25(OH)D concentration, and the treatment was more than three times more effective at mobilizing and storing in fat tissue than the same dosage of vitamin D_2 by the end of the study. Only two studies found no significant differences between vitamin D form and serum 25(OH)D concentration (Holick et al., 2008; Biancuzzo et al., 2010).

Breast fed infants receive vitamin D from three main sources which aids in meeting their needs for rickets prevention: that which is supplied through the placenta during pregnancy which is related to mother’s status, breast milk, and sun exposure (Hollis and Wagner, 2004). Due to limited sun exposure in infancy and the prevalence of vitamin D deficiency/insufficiency of mothers and infants in North American (Ward et al., 2007), Health Canada currently recommends that breast fed infants receive 400 IU (10 μg) /day of supplemented vitamin D. Some earlier studies have reported a low transfer of vitamin D metabolites from the mother’s circulation to breast milk (Hollis et al., 1981; Hillman, 1990), while others claim this is due to poor maternal status as a result of insufficient dietary intake. When status is optimal (not readily agreed upon in the scientific community), vitamin D transfer will meet infant needs (Hollis and Wagner, 2004; Hollis et al., 2015). In order for infants ages 0-12 months to meet the Adequate Intake (AI) of 10 µg (400 IU)/day as well as achieve 'normal' vitamin D status, it has been estimated that lactating women would have to consume approximately 100 µg (4000 IU)/day-160 µg (6400 IU)/day or greater (which is five or more times the current recommendation) (Hollis and Wagner, 2004; Thiele et al., 2013). The CPS has increased this estimation to recommend 20 µg (800 IU)/day for infants living above 55° latitude in winter months. Infant formulas in Canada must be fortified with vitamin D and food labels state that those consuming less than 1L/day would require additional supplementation (reviewed by Godel, 2007).

1.6.9 Shortcomings/gaps in the literature on estimating calcium and vitamin D intake

1.6.9.1 Estimating dietary intake: methodology

There is limited research on dietary and supplement intake of women in North America (including Canada) and Europe in the early stages of pregnancy through the third trimester and beyond to postpartum, thus there have been few methodology validation studies in this population. It is uncertain whether the tools (24-hour recall, FFQ, and food diaries) used to estimate calcium and vitamin D intake would be just as valid in a pregnant or lactating population (Rifas-Shiman et al., 2006; Oken et al., 2007). In the general population, calcium intake studies will often use four to seven day food diaries as the benchmark when comparing and validating FFQ instruments as food diaries are thought to be the most accurate measurement of estimated dietary intake (Cummings et al., 1987; Blalock et al., 2003; Sebring et al., 2007; Hacker-Thompson et al., 2009). Some calcium intake FFQs only evaluate intake from major sources of calcium, while others are more detailed and contain longer lists of food and beverages containing calcium. A calcium FFQ validation study where 75% of participants were female with a mean age of 38 ± 11 years old compared three FFQs (Diet History Questionnaire (DHQ), Calcium Questionnaire (CQ), and Short Calcium Questionnaire (SCQ)) varying in length to a seven day food diary. The estimated mean calcium intake measured from the DHQ which was the longest in length and measured other micronutrients besides calcium, resulted in an estimated daily calcium intake that was significantly lower ($p < 0.001$) than the seven day food diary, whereas the CQ which measured only calcium intake, resulted in an estimated intake that was significantly higher ($p < 0.001$) than the seven day food diary. Correlations based on linear regression found all three instruments (DHQ, CQ, and SCQ) did not correlate well to the seven day food dairy ($r^2 = 0.21, 0.33, 0.37$, respectively) (Sebring et al., 2007). On the contrary, two studies showed that FFQs were reliable and valid methods used to estimate calcium intake, at least in the general population (Cummings et al., 1987; Blalock et al., 2003). The FFQs in both studies were validated against seven day food diaries and were highly correlated ($r = 0.76, 0.72$, respectively) (Cummings et al., 1987; Blalock et al., 2003). However, the estimates obtained from the FFQ were from predominately women of childbearing age and were somewhat inconsistent as some were reported to significantly underestimate calcium (Sebring et al., 2007; Hacker-Thompson et al., 2009) while others were found to overestimate calcium intake (Cummings et al., 1987; Blalock et al., 2003) when compared to food records. Bland-Altman analysis can be also be used to validate two different dietary collection methods and is thought be better at assessing the comparability of the instruments than correlation or regression analysis. Bland-Altman analysis measures the difference between the two dietary collection methods compared to the average of the two methods (Giavarina 2015). Using this analysis, Hacker-Thompson et al. (2009) found agreement in a calcium FFQ used when compared to a three day food dairy. In conclusion, in reviewing the literature, FFQs, regardless of format (computerized vs. paper), comprehensiveness (34-124 items), and the way food items were measured (descriptive vs. household measurements) may not be an accurate way of estimating calcium intake. Additionally, FFQs are designed to capture usual intake and food intake changes over the course of pregnancy making it difficult for FFQs to capture actual intake.

Twenty-four hour recalls estimate the quantity of food consumed from the previous day but are only as reliable as the participant's ability to quantify common household measurements, recall accurately what was consumed, and be representative of 'usual intake' within the desired time period. Doubly labeled water (DLW) studies reviewed by Subar et al. (2015) measured energy intake more accurately than a 24-hour recall. It was reported that estimated energy intake from 24-hour recalls of young to middle age women of childbearing age were underreported by 6-16%, using doubly labelled water as a measure of 'actual energy' intake (Subar et al., 2015). Since there is a positive association between estimated dietary calcium and energy intake, energy adjusted intake was used to assess micronutrient changes (Rifas-Shiman et al., 2006; Durham et al., 2011). It has been suggested that the reliability of a 24-hour recall for estimating an individual's calcium intake can be improved by increasing the number of recalls from three to four individual days (Rush and Kristal, 1982). Also, combining methods of data collection (short-term and long-term) has been suggested to help improve the precision of estimating dietary intake (Subar et al., 2015). Twenty-four hour recalls are more suitable for studying large populations as they are quicker to administer and require less resources to analyze than food records. However they should be validated for the specific population studied (Castell et al., 2015). As long as researchers are aware that self-reported dietary intake like those collected in dietary surveillance surveys like NHANES and CCHS have limitations that over and underestimate caloric, micro, and macronutrient intake, they can still provide a reasonable estimation of dietary consumption in large cohorts (Subar et al., 2015).

1.6.9.2 Estimating dietary intake from calcium and vitamin D in pregnancy: lack of comprehensive data on intake through pregnancy and postpartum

In the literature, researchers often combine trimesters where intake could vary significantly or take one measure to represent all of pregnancy. For example, Haugen et al. (2008) only looked retrospectively at dietary intake from the first and second trimesters only. Derbyshire et al. (2009) studied women during each trimester, but the study was limited by small sample size (n= 42) that completed each phase, and they did not collect supplement intake. Viljakainen et al. (2010) used a FFQ at 35 weeks gestation and made the assumption that this single measure was representative of intake throughout pregnancy.

Within the last twelve years, there has been an increase in calcium and vitamin D fortified food and beverage production as well as consumption (Harville et al., 2004). Fortified milk, margarine, cheese, yogurt, orange juice, bread and breakfast cereals, and butter represent the major sources of calcium and vitamin D in the North American diet (reviewed by Calvo et al., 2005). FFQs like those used in the Cummings et al. (1987) study did not have to include fortified sources of calcium and vitamin D like protein bars, tofu, cereals, fruit juices, plant-based beverages, and waffles because these products did not exist. More recent studies (Rifas-Shiman et al., 2006; Oken et al., 2007; Mannion et al., 2007; Haugen et al., 2008; Derbyshire et al., 2009) did not identify if participants were asked about fortified foods and beverages when filling out 24-hour recalls, food diaries, or FFQs which may underestimate calcium and vitamin D intake in pregnant and postpartum women. A review paper that compared countries that require mandatory fortification like Canada found that the general population consumed 2-3 μg

(80-120 IU) more vitamin D/day with the addition of vitamin D fortified products than those countries with optional and no food/beverage fortification showing how much greater estimated dietary intake would be with the addition of fortified foods and beverages (reviewed by Calvo et al., 2005). A more recent study using NHANES data of persons ≥ 2 years old, assessed micronutrient intake from naturally occurring and fortified sources and found that estimated naturally occurring estimated dietary intake of vitamin D was 1.9 µg (76 IU) ± 0.4 µg (16 IU)/day whereas fortified intake was 2.9 µg (116 IU) ± 0.1 µg (4 IU)/day. Estimated calcium intake from naturally occurring sources was 885 ± 12 mg/day while fortified sources only contributed an additional 55.3 ± 2.5 mg/day (Fulgoni et al., 2011). This study did not indicate if fortified beverages (other than vitamin D fortified milk) were included in the analysis which may have led to an under estimation of actual intake. Thus, vitamin D intake may not be as low as what has been reported in the earlier literature.

1.6.9.3 Infant feeding modality and its impact of estimated calcium and vitamin D intake

Very little is also known about dietary and supplemental calcium and vitamin D intake for women who exclusively breast feed during the postpartum period and even less is known about women who exclusively use infant formula or mix feed. Since maternal vitamin D intake influences breast milk composition and calcium intake impacts maternal bone health, it would be important to understand if intake varies in these three different feeding paradigms. Two studies that were reviewed for this thesis were of women in the postpartum period but neither described whether participants breast fed or not (Rees et al., 2005; Derbyshire et al., 2009). In a study of overweight and obese postpartum women (n= 450) that breast fed, mixed fed, or formula fed, all groups had greater than half of participants meet less than 50% of the AI at the time for calcium

(1000 mg). Formula feeders were less likely to take a vitamin or mineral supplement, and in the mixed feeders and formula feeders groups, greater than half of women had estimated intakes that fell below 50% of the AI for vitamin D which as 5μg (200 IU)/day at the time of the study. Mean estimated dietary calcium intake for the breast feeding group was 1029 ± 35 mg/day, 814 ± 39 mg/day for the mixed feeding group, and 670 ± 35 mg/day for the formula feeding group. The mean estimated dietary vitamin D intake was 6 μg (240 IU) ± 0.4 μg (16 IU)/day for the breast feeding group, 5 μg (200 IU) ± 0.4 μg (16 IU)/day for the mixed feeding group, and 4 μg (160 IU) ± 0.4 μg (16 IU)/day for the formula feeding group. However when controlling for co-variables, there were no significant differences in individual nutrient intake for the three groups. All groups reported what was defined as poor diet quality (high intake of processed grains, sweetened beverages including soda, and desserts) (Durham et al., 2011). Whether or not these results can be translated to a healthier population is questionable since there are additional issues with the obese population in the literature like underreporting food and caloric intake (Braam et al., 2001; Durham et al., 2011). To determine over/underestimation of dietary intake, studies have used DLW and changes in body weight (Subar et al. 2015). For a pregnant population, weight gain during gestation could be compared to IOM standards. One would predict from Mannion et al. (2007) where breast feeding women (mean BMI: 23.0 ± 3.4) were meeting the EAR for both nutrients with diet and supplements when not restricting milk consumption, that breast feeding women (either exclusive or non-exclusive) might consume greater amounts of these two nutrients than women that exclusively formula feed. Understanding intake of women with different infant feeding modalities would help to develop target interventions for the group(s) at greatest risk.

1.6.9.4 Contribution of supplements to intake during pregnancy and the postpartum period

Few studies reported the use of calcium and vitamin D supplements across pregnancy and the postpartum period. Usually only intake from diet or total intake was reported. This may be a significant limitation with estimating vitamin D intake since the majority of pregnant women's intakes comes from supplements (Rifas-Shiman et al., 2006; Haugen et al., 2008). Mannion et al. (2007) only looked at calcium and vitamin D intake during lactation and found that despite the large contribution of vitamin D containing supplements to overall dietary intake, women who restricted milk (< 250 mL/day) on average consumed 6.1 µg (244 IU) ± 4.7 µg (188 IU)/day, and therefore did not meet the EAR.

1.6.9.5 Considering vitamin D intake when estimating calcium adequacy

All the studies reviewed that reported calcium intake failed to identify women at risk for calcium deficiency using the criteria of meeting current recommendations for calcium but not for vitamin D. Both calcium and vitamin D intake should be considered when identifying those at risk for calcium inadequacy due to lower calcium intake as well as impaired absorption and metabolism. Those at greatest risk might be those that do not meet the EAR for either nutrient. It is uncertain how many pregnant or postpartum women fall into this high risk category.

1.6.9.6 Conclusion

In conclusion, calcium and vitamin D are two important nutrients for both the pregnant woman and fetus as well as the lactating woman and the breast fed infant. Inadequate vitamin D status in a pregnant woman has been determined by measuring serum 25(OH)D concentrations, and this has been associated with an increased risk of adverse pregnancy outcomes such as preeclampsia, GDM, poor fetal skeletal growth, low birth weight, and reduced bone mass (Haugen et al., 2008; Derbyshire et al., 2009; reviewed by Hacker et al., 2012; Olausson et al., 2012; Lacroix et al., 2014). Insufficient calcium intake in a pregnant woman results in increased maternal bone loss (Thomas and Weisman, 2006; Kalkwarf, 1999; Ross, 2011) as well as increased adverse pregnancy outcomes such as preeclampsia and preterm labor (reviewed by Hacker et al., 2012; Olausson et al., 2012).

A dietary intake assessment of calcium and vitamin D is one way of identifying individuals as well as populations at risk for not meeting the RDA and EAR, respectively. Specifically, 24-hour recalls while not the most accurate in estimating dietary intake are useful when assessing large cohorts and aid in identifying dietary intake trends. The accuracy of 24-hour recalls can also be increased with better estimations of portion sizes, using a Multiple-Pass Method, combining various dietary assessment methods, and increasing the number of days assessed. To determine the validity of one dietary assessment tool to another, correlation, regression, and Bland-Altman analysis can be used. A review of the current literatures concludes that women are meeting the recommended guidelines for calcium intake with diet alone during pregnancy, however, whether they are meeting those guidelines in the postnatal period remains unclear. Pregnant women are consistently reported to not meet the recommended guidelines for vitamin D with diet alone and rely heavily on supplement usage to meet current recommendations. Only one study by Mannion et al. (2007) was identified as assessing total vitamin D intake during lactation of healthy women, and they reported that only women that drank milk (> 250 mL/day) met recommendations. According to What We Eat in America, NHANES 2005-2006, non-pregnant women of child bearing age consume approximately 160 mL of milk/day which can put them at risk for poor vitamin D intake prior to pregnancy. From the literature, several variables have been identified that influence a pregnant and lactating women's ability to meet recommendations. These include age, smoking status, socioeconomic status, parity, pre-pregnancy BMI, weight gain during pregnancy, supplement usage, and milk drinking status. There are also several gaps in the literature that remain including identifying dietary patterns of intake, the assumption that one measure of intake during pregnancy is representative of all three trimesters and postpartum, the contribution of fortified food to dietary intake, considering vitamin D intake when determining calcium adequacy, and assessing intake based on breast feeding status, supplement usage, milk drinking status, and other co-variables. To better plan for counselling and publish health recommendations, a study is needed to determine if Albertan women are meeting the DRI for calcium and vitamin D with diet and supplementation during each trimester of pregnancy as well as postpartum as well as identify the characteristics associated with reduced intake.

Chapter 2: Research plan

2.1 Statement of problem

Assuring an 'adequate' dietary intake of calcium and vitamin D is essential for optimal maternal and infant health. It is uncertain whether women in Alberta are meeting calcium and vitamin D dietary recommendations during each trimester of pregnancy as well as postpartum due to studies using single estimates during pregnancy, combining intake from different trimesters, and not considering the contribution of supplements to total intake. Without total intake, one cannot identify those at risk for calcium inadequacy due to suboptimal calcium intake, vitamin D intake, or both. The major sources of calcium and vitamin D in the diet of pregnant or lactating women are not known, nor the contribution of fortified foods and beverages as well as supplements. This information is needed to target interventions to improve the diet quality of pregnant and postpartum women.

2.2 Rationale

Dietary quality during pregnancy is vital to the mother's nutritional status and the fetus's growth and development in utero. A maternal diet insufficient in calcium can increase the risk for gestational and long term complications such as preeclampsia, preterm delivery, and excessive bone loss (reviewed by Hacker et al., 2012). There has been an overall upward trend in the rate of preterm births in Alberta from 8.3 per 100 live births in 2001, 9.1 in 2005, and 8.7 in 2010 (Alberta Reproductive Health Report Work Group- Government of Alberta, 2011). A diet insufficient in vitamin D has been associated with preeclampsia, GDM, poor fetal skeleton growth, reduced gestation, lower birth weight and size, and reduced bone mass and length throughout life (Haugen et al., 2008; Derbyshire et al., 2009; reviewed by Kaludjerovic and Vieth, 2010; reviewed by Hyppönen, 2011; reviewed by Hacker et al., 2012, Lacroix et al., 2014). Current studies on the fetal environment suggest that the vitamin D status of the mother could possibly impact immune system functioning later in life of the offspring and make him/her more prone to CVD, T1DM, asthma, and allergies (Valjakainen, 2010; reviewed by Hyppönen, 2011).

Especially in the first six months when breast milk is the infant's sole source of nutrition, it should provide 100% of the infant's nutritional needs. It is vital to ensure that women consume adequate calcium and vitamin D in their diet prior and during pregnancy so breast milk has adequate content to support the infant while also supporting their own status and prevent bone loss above and beyond the normal loss from breast feeding.

From the literature, it is known that during pregnancy, the majority of calcium comes from the diet and vitamin D comes from supplements. Those at risk for not meeting the DRI for calcium include non-Caucasians, smokers, increased parity, young age, high BMI, low caloric intake, poor diet quality (micro and macronutrients alike), less education, low socioeconomic status, those not taking dietary supplements, and non-milk consumers. Similarly, those at risk for not meeting the DRI for vitamin D included smokers, those with low caloric intake, young age, high BMI (classified as overweight or obese), less education, and multiparity.

Although a high pre-pregnancy BMI is associated with low calcium and vitamin D intake, it is not known if there is an association with weight gain during pregnancy and intake of these two micronutrients. Since many studies have not looked across pregnancy or have not measured or reported supplement intake, it is uncertain whether Albertan women are meeting the EAR for calcium and vitamin D in each trimester of pregnancy and postpartum or if there are any significant differences in intake between trimesters. Little is also known about the contribution of fortified foods and beverages (other than milk) to overall dietary intake. There also is limited data from North America to describe the dietary patterns of these women and the sources of calcium and vitamin D in their diet during pregnancy and in the postpartum period. Lastly, the actual risk for calcium deficiency has not been explored since this includes women who are not meeting the recommendations for calcium, vitamin D, or both. In conclusion, it is important to gain understanding on estimated calcium and vitamin D intake during each trimester and postpartum to identify those time points where women are at the greatest risk. It is also important to identify dietary patterns and characteristics of women who are at the greatest risk for not meeting the DRI.

2.3 Research objectives/questions

The goal of this research was to describe calcium and vitamin D intake and the sources of these two nutrients in the APrON cohort. It should be noted that calcium was the primary nutrient studied and vitamin D was included in the scope of this research study due to its vital role in calcium absorption.

The objectives of this research were to:

1. Describe calcium and vitamin D intake across the three trimesters of pregnancy as well as 3 months postpartum in a large cohort of women in Alberta (APrON)

The following research questions will be addressed specific to this objective:

   a. Are there significant differences in estimated mean intake between the each trimester of pregnancy and 3 months postpartum for supplemental, dietary, and total intake of calcium and vitamin D?
   b. Do women's estimated mean total intake of calcium and vitamin D change over time?

   c. What proportion of women are meeting the EAR for calcium and vitamin D during pregnancy and 3 months postpartum?

2. Identify women who are at risk for not meeting recommendations for calcium and vitamin D during pregnancy and 3 months postpartum based on milk drinking status and supplement use

The following research questions will be addressed specific to this objective:

   a. What is the estimated mean intake of calcium and vitamin D of the four categories of milk drinkers and do these differ across pregnancy and 3 months postpartum?

   b. How much more likely are women who are milk drinkers to meet the EAR for calcium and vitamin D than non-milk drinkers?

   c. What is estimated mean calcium and vitamin D intake based on supplement use?

   d. What proportion of women are meeting the EAR based on calcium or vitamin D supplement use?

   e. What is the likelihood of meeting the EAR for calcium or vitamin D if women take a calcium or vitamin D containing supplement?

3. Describe the contribution of calcium and vitamin D fortified foods and beverages in meeting recommended guidelines (including milk)

The following research question will be addressed specific to this objective:

   a. What is the contribution of fortified foods and beverages to overall calcium and vitamin D intake?

4. Identify the proportion of women who may be at risk for calcium malabsorption and/or low calcium intake (defined as intake below the EAR)

The following research questions will be addressed specific to this objective:

a. What proportion of women are at risk for decreased calcium absorption or intake as a result of not meeting the EAR for one or both of these nutrients?

b. What is the relationship between calcium and vitamin D intake during pregnancy and 3 months postpartum?

c. What are the odds of women meeting the EAR for calcium and vitamin D?

5. Describe calcium and vitamin D intake related to macronutrient and caloric consumption

The following research question will be addressed specific to this objective:

a. Do women that meet the EAR for calcium or vitamin D consume greater amounts of macronutrients than those that do not meet the EAR?

b. How does caloric intake influence calcium and vitamin D intake?

6. Describe calcium and vitamin D intake related to sociodemographic status

The following research questions will be addressed specific to this objective:

a. What are the characteristics (income, parity, marriage, race, etc.) of women meeting the EAR for calcium and vitamin D vs. not?

b. What proportion of women are meeting the EAR based on infant feeding status?

c. Are women who breast feed more likely to meet the EAR for calcium and vitamin D than women who formula feed or combination feed?

d. Is there an association between women exceeding gestational weight gain guidelines during pregnancy and not meeting the EAR for calcium and vitamin D?

Chapter 3: Methods

3.1 Study design and subjects

The combined first and second cohorts (n= 1188) of the Alberta Pregnancy Outcomes and Nutrition (APrON) study were used for this research. Pregnant women from Edmonton and Calgary, Alberta, Canada and the surrounding areas were recruited from May 2009 to November 2010 (Manca et al., 2013). Recruitment occurred at obstetric, family medicine, and ultrasound clinics with the assistance of the Women and Children's Health Research Institute (WCHRI) at the University of Alberta. Other recruitment tactics included posters in areas frequented by pregnant women such as grocery stores, community centers, prenatal education classes, baby fairs, as well as advertisements on television and radio. The inclusion criteria included ≥ 16 years old, ≤ 27 weeks gestation upon entry into the study, and ability to answer questions in English. Women interested in participating were contacted to explain the study in greater detail, and if interested, were scheduled clinic visits. The study received approval from the University of Calgary Health Research Ethics Board as well as the University of Alberta Health Research Ethics Biomedical Panel. All women provided informed consent prior to being enrolled in the APrON study (Kaplan et al., 2014).

3.2 Data collected from the APrON study used to address thesis objectives

Recruited women who met the inclusion criteria were assessed for calcium and vitamin D intake from diet with a 24-hour food recall questionnaire using a Multiple-Pass Method that was administered by a trained nutrition personnel or Registered Dietitian (RD) during each trimester of pregnancy (time points (TP)-A, B, and C) and 3 months postpartum (TP-E). Dietary food models were utilized to help increase accuracy of serving size estimates. The trained nutrition professional or RD also used probing tactics to obtain further information on cooking methods, meal times, food brands, as well as completeness of recall. For example, if a participant stated they had cereal for breakfast, the nutrition professional would clarify if they had dry cereal or cereal with milk or a milk alternative. Once the recall was obtained, it was read back to the participant for accuracy and comprehensiveness. Several of the women entered the study in the second trimester so dietary data was only collected for three time points (Kaplan et al., 2014).

Calcium and vitamin D supplementation usage from a multivitamin/mineral or single supplement was measured using a Supplement Intake Questionnaire (SIQ) designed specifically for the APrON study (Gómez et al., 2013). The SIQ was based on previously validated questionnaires used in the National Cancer Institute's Diet Questionnaire, the NHANES Dietary Supplement Use Questionnaire (2005-2006), as well as the CCHS Survey (2004) that were modified for a pregnant population (Gómez et al., 2013). The SIQ was initially used in a pilot study which consisted of fifty women in the APrON study during their first and second visit to assess efficacy and detail of information obtained. The SIQ was administered by trained nutrition personnel at each trimester visit and 3 months postpartum. The SIQ inquired about brand name, strength, frequency of use, duration, and dosage. Since the SIQ elicited when the women began taking the supplements, those that were recruited at 14-26 weeks were able to provide supplement intake retrospectively for the first trimester. At follow up visits, if women had begun or stopped supplements since their last visit, these adjustments were also made to their supplement intake.

3.3 Collection of co-variables

Pre-pregnancy information collected during the first visit included past medical history, socio-demographics (such as age, marital status, household income/year, education level, and ethnicity), physical activity, and BMI. These and several co-variables such as parity, planned pregnancy, use of fertility treatment, and tobacco and alcohol use during pregnancy were identified as potentially influencing calcium and vitamin D intake. A smoker or alcohol drinker was defined as a person who was currently using the substance. Daily users were not differentiated from the more occasional user. During the 3 months postpartum visit, participants were asked about infant feeding practices and whether they breast fed, formula fed, or used a combined regimen. To be considered a combination feeder, a breast feeding woman had to use formula at least weekly. If a women had changed feeding modalities, which was common since women often start supplementing with formula until their milk comes in, the classification that was followed for the greatest length of time was used in this study.

Physical activity was assessed during each trimester as well as 3 months postpartum using a validated short questionnaire which included on the job activity, cardiovascular fitness, weight training, and recreation (Baecke et al., 1982; Kaplan et al. 2014). Only routine cardiovascular fitness was considered for this study since the American College of Obstetrics and Gynecology recommends thirty minutes or more of moderate exercise per day on most if not all days of the week for women 19-50 years old. The College further states that, "healthy women should get at least 150 minutes per week of moderate intensity aerobic activity such as brisk walking during and after their pregnancy." Therefore, a participant was identified as an aerobic exerciser if she exercised moderately for ≥ 2.5 hours/week. If data was missing to indicate type of exercise, duration, or the intensity of physical activity, the participant was excluded from the statistical analysis.

3.4 Estimation of milk intake in the cohort

A serving of milk was defined as 250 mL (1 cup) according to Canada's Food Guide. The total amount of milk consumed was calculated for each time point. Milk consumers were identified by reviewing all the 24-hour recalls that contained the words ‘milk, fluid’. The APrON women were divided into four categories which represented approximate quartile intakes: high milk drinkers (> 500 mL/day) which was also the recommendation in Canada’s Food Guide, medium (250.1-500 mL/day), low (1-250 mL/day), and no milk (0 mL/day) to determine if a higher intake of milk increased the likelihood of meeting dietary guidelines than those that consumed no milk. Since all fluid milk requires vitamin D fortification under the Health Canada’s Food and Drug Act, buttermilk and evaporated milk were also included in the analysis. Although condensed milk is vitamin D fortified, it was not included in the analysis since it is dissimilar in nutrient content to other milks due to its added sugar content and usage in baking. Milk found in mixed dishes such as casseroles or mashed potatoes were not included since it was difficult to discern the source of calcium and vitamin D in those types of dishes.

3.5 Determination of weight gain during pregnancy in the cohort

Upon entering the study, women provided a stated pre-pregnancy weight and height so BMI could be calculated. Women were subsequently weighed at each follow up visit. Weight gain during pregnancy was determined by finding the difference between the highest weight in pregnancy and the pre-pregnancy weight. The third trimester weight was used if the highest weight was not obtainable or it was greater than the highest weight obtained during pregnancy to allow for a greater number of participants to be included. Women were categorized as exceeding, meeting, or below the IOM and Health Canada guidelines for gestational weight gain as shown in **Table 3.1** based on their BMI status.

Table 3.1: Target weight gain according to BMI status

BMI status (kg/m^2)	Weight gain guidelines (lbs. & kg)
Underweight (< 18.5)	28-40 lbs. (12.7-12.2 kg)
Normal (18.5-24.9)	25-35 lbs. (11.4-15.9 kg)
Overweight (25-29.9)	15-25 lbs. (6.8-11.4 kg)
Obesity (> 30)	11-20 lbs. (5-9.1 kg)

Source: http://www.hc-sc.gc.ca

3.6 Estimation of adequate intake for calcium and vitamin D

The Estimated Average Requirement (EAR) was used to determine whether women were meeting or not meeting the recommendations for calcium and vitamin D during pregnancy and postpartum. The EAR, which is 800 mg and 10 µg (400 IU)/day, respectively, was used if population intake was being described. The RDA which is 1000 mg/day for calcium and 15 µg (600 IU)/day for vitamin D during pregnancy and lactation was used if describing the intake of the individual. In a review by Trumbo et al. (2013) on the proper use of the DRI, the proportion of women below the EAR represented those at risk for inadequacy. Women were also identified who were exceeding the Upper Limit (UL) of 2500 mg of calcium/day and 100 µg (4000 IU) of vitamin D/day.

3.7 Cleaning of database and estimation of daily dietary intake of calcium and vitamin D

If a woman had dietary intake data for at least one of the four time points, they were included, making 3023 records and 1048 women available for this study. The first step in cleaning the dietary data was to remove the 24-hour recalls that were outliers which were defined as reported calorie and/or fiber intakes that were ± 3 standard deviations (SD) from the mean. This resulted in the removal of 46 dietary records, making 2977 dietary records and 1046 women out of a possible 1048 women available for this thesis. Data was then cleaned by this writer for calcium and vitamin D following similar principles. More specifically, dietary intake outliers for calcium and vitamin D were first flagged if they were ± 3 SD from the mean. Before dietary data was eliminated, the daily estimates were verified by going back to the actual 24-hour recalls to ensure that intake data was coded appropriately. No women were removed as it was possible to have low and high intakes of these nutrients, and the literature has reported that many women consumed increasing amounts of dairy from the first to the second trimester (Rifas-Shiman et al., 2006).

The next process used was setting a low intake threshold/triggers for data review and cleaning. This was set as a calcium intake < 500 mg/day and vitamin D intake < 100 IU/day. All participants were reviewed if consuming < 1000 kilocalories (kcal)/day as well. If participants met these criteria (which 550 participants did), each of the actual 24-hour recalls were reviewed for accuracy and only removed if one of the following criteria were met: the woman had indicated to the research assistant that the intake was not usual due to illness (other than morning sickness as indicated on recall), there was an incomplete dietary recall, the woman reported she had an atypical eating pattern on the day of the recall, or the recall contained only one meal which was not observed in any of the other dietary recalls from the other time points. Based on these criteria, dietary data was removed for 1 participant in time point A (TP-A), 7 participants in time point B (TP-B), 1 participant in time point C (TP-C), and 2 participants in time point E (TP-E). This left 2966 recalls and no further removal of participants (1046) in the database.

Lastly, since the contribution of fortified foods and beverages was of interest in this study, each 24-hour food record that contained the following products: orange juice, soy beverage, rice beverage, almond beverage, vitamin water, and tofu were compared to what was entered in the dietary intake profile of the participant. These products were chosen as these food and beverage items often have fortified and non-fortified varieties making it easy for them to be coded incorrectly. No participants were removed, however, several dietary recalls were re-coded to reflect the use of fortified foods as indicated on the 24-hour recall. The final number of dietary records used in this study are in **Table 3.2**. Note, there was more supplement data in TP-A than dietary data because this information was collected retrospectively at TP-B particularly when subjects entered the APrON study in the second trimester.

Table 3.2: Number of participants post data removal

Time points	**Diet**	**Supplements**	**Total**
1st trimester (TP-A)	n= 236	n= 832	n= 232
2nd trimester (TP-B)	n= 989	n= 1047	n= 984
3rd trimester (TP-C)	n= 885	n= 921	n= 869
3 mos. postpartum (TP-E)	n= 859	n= 867	n= 828
Completed all time points (and contains total intake)			n= 159

3.8 Estimation of total calcium and vitamin D intakes

Total dietary intake was estimated for calcium and vitamin D from the 24-hour recall as well as the SIQ collected from each woman during pregnancy (one to three times) and 3 months postpartum. Estimated total intake could only be calculated if dietary and supplement intake were known. The 24-hour recall data was entered into Food Processor (version 10.6; ESHA

Research, Salem, OR, USA), a food analysis software, to calculate macronutrient and micronutrient content from reported food and beverage intake.

3.9 Collection, cleaning, and estimating daily supplement intake of calcium and vitamin D

During each trimester visit and 3 months postpartum, the trained nutrition professional filled out the SIQ with each participant. The questionnaire consisted of commonly used supplements in three major categories: multivitamin, single supplement, and herbs. Each category contained an “other” box so the nutrition professional could record other supplements taken. To aid in recall accuracy of Natural Health Products (NHPs), participants were asked to bring in the bottles of the supplements they consumed from home. Each bottle contained the Natural Product Number (NPN) that is linked to Health Canada’s Licensed Natural Health Products Database (LNHPD) (Gómez et al., 2013). If labels were not brought in, the women provided the supplement’s brand name and dosage for easy retrieval of the NPN. When a nutritional supplement could not be found in the LNHPD, the manufacturer’s website was used to retrieve nutritional information. During the first visit, all women were asked about supplement use (frequency and dosage since becoming pregnant) and in subsequent visits, they were asked about any changes to their supplement use since the last visit as well as their current intake. Supplement intake was identified by the NPN, and a NHP database was created for this and other APrON studies which linked the NPN to the nutrient content of each supplement. Supplements that were not in the LNHPD were provided their own unique code for identification purposes. To date, there are over 900 supplements used by the women enrolled in the APrON study. The most common NHPs were used as a default if the nutrient content of the supplement was unknown or if not enough information was provided about the supplement.

A detailed conversion method was developed for this thesis by this author as to not overestimate intake if participants had taken a supplement for a fraction of the time point or if they switched supplements at any point during that time period. Corrections were applied based on daily use (days/week) and trimester (weeks/trimester). For example, a calcium supplement would be reported as such: Lifebrand 650 mg taken twice a day (BID), six days a week, for five weeks of the first trimester. In this example, one would multiply the dose of 1300 mg by 0.86 representing the conversion factor for days/week and 0.38 representing the conversion factor of weeks/trimester to get 424.84 mg/day of calcium. With this method, the following major assumptions were made: each trimester was approximately thirteen weeks, a month was approximately four weeks, and supplements were taken the entire trimester unless otherwise specified.

Calcium and vitamin D intake from supplements were reviewed for accuracy by comparing the actual SIQ to the data entered. Also, all '0' values were verified with the SIQs to make sure these were actual 'no intake' rather than missing time points. Outliers of calcium and vitamin D were identified as > 2500mg/day and 4000 IU/day, respectively, which are the ULs for these nutrients. Intakes which met this criteria were chosen at random for comparison with the SIQ to ensure accuracy of data entry. However, it was plausible that women could exceed the UL by combining multivitamin with single supplement use, so these intakes were not removed.

3.10 Statistical analysis

Estimated nutrient intake was calculated and analyzed using Stata Version 11.2. A p value < 0.05 was considered significant. The data was analyzed for normality using a Kernel density estimated test. Mean ± SD was used to describe intake. The differences in dietary intake, supplement, and total intake of calcium and vitamin D among the three pregnancy time points and 3 months postpartum were determined using a Kruskal-Wallis equality-of-populations rank test and post-hoc estimation using a Two-sample Wilcoxon rank-sum test. Odds ratios (OR) with 95% confidence intervals (CI) were used to identify how much more likely women (with certain characteristics) were to meet nutrition recommendations compared to another. Fisher's exact chi-square test was used in identifying covariate influence on nutrient intake. A one-way ANOVA was used to compare dietary and total intake of SU and NSU. This will be discussed with the research results in further detail.

Chapter 4: Results

4.1 Subjects

The baseline characteristics of the participants (n~1054) are presented in **Table 4.1**. Most of the women in this cohort were older (mean: 31 ± 5 years old), had a healthy BMI status (mean: 24.2 ± 4.7), a minimum of trade school or university education (90%), Caucasian (82%), did not smoke (97%), did not drink alcohol during pregnancy (82%), were married/common-law partnership (96%), had a planned pregnancy (81%), and did not use fertility treatment (92%). Approximately half of the cohort was nulliparous (56%), had a household income greater than \$100,000 (CAD)/year (59%), and participated in regular aerobic exercise during pregnancy (defined as moderate exercise ≥ 2.5 hours/week) (53%). **Table 4.2** represents characteristics of the women measured at 3 months postpartum. During that period, 68% of the cohort reported to exclusively breast feed. Approximately half of the cohort exceeded desirable weight gain during pregnancy as recommended by the IOM and Health Canada (52%) and reported they did not participate in regular aerobic activity (58%).

Table 4.1: Demographic and BMI characteristics of women (n~1054) enrolled in the APrON cohort at baseline

Characteristic	**%**
Age (years)	
17-30	43%
31-45	57%
Pre-pregnancy BMI (kg/m^2)	
underweight (< 18.5)	4%
normal (18.5-24.9)	63%

overweight (25-29.9)	21%
obese (> 30)	12%
Household income (CAD)/year	
> $100K	59%
$70-99.9K	22%
$40-69.9K	13%
$20-39.9K	4%
$ < 20K	2%
Highest education	
post university	22%
trade/university	68%
< high school/high school	10%
Ethnicity	
Caucasian	82%
Non-Caucasian	18%
Smoking	
yes	3%
no	97%
Marital status	
married/common-law	96%
single	3%
separated/divorced	1%

Ethanol use (during pregnancy)	
yes	18%
no	82%
Parity	
nulliparous	56%
parous	44%
Planned pregnancy	
yes	81%
no	19%
Fertility treatment	
yes	8%
no	92%
Aerobic exerciser (during pregnancy)	
yes	53%
no	47%

Table 4.2: Maternal characteristics at 3 months postpartum in the APrON cohort

Characteristic	**%**
Infant feeding modality	
breast feeding	68%
combination feeding	23%

formula feeding	9%
Compliance with gestational weight gain guidelines	
below	15%
meeting	33%
exceeding	52%
Aerobic exerciser (postpartum)	
yes	42%
no	58%
Ethanol use (postpartum)	
yes	51%
no	49%

4.2 Estimated calcium and vitamin D intake

Figure 4.1 illustrates estimated mean calcium intake during pregnancy and 3 months postpartum from food and beverages (diet), supplements, and total intake (food/beverages + supplements). When comparing the differences between mean intakes using a Two-sample Wilcoxon rank-sum test ($p< 0.05$), there was a significant increase in estimated dietary calcium intake from TP-A to TP-B, no significant change from TP-B to TP-C, and a significant decrease from TP-C to TP-E. There were no significant differences in supplement intake during TP-A and TP-E. Supplements made a small contribution to total intake and did not differ between time points; the differences across time points for total intake was the same as dietary intake. The highest proportion of women below the EAR for total calcium and having the potential for inadequacy during pregnancy occurred at TP-A (16% not meeting the EAR). Of note, 29% of women were not meeting the RDA. At TP-E, 20% of women were not meeting the EAR. The range of those exceeding the UL was small with only 2-6% during pregnancy and 3% at 3 months postpartum. Calcium intake came predominately from foods and beverages during pregnancy and postpartum in this cohort (71-88% of total intake) and women met the EAR with diet alone.

Figure 4.1: Estimated calcium intake during pregnancy and postpartum

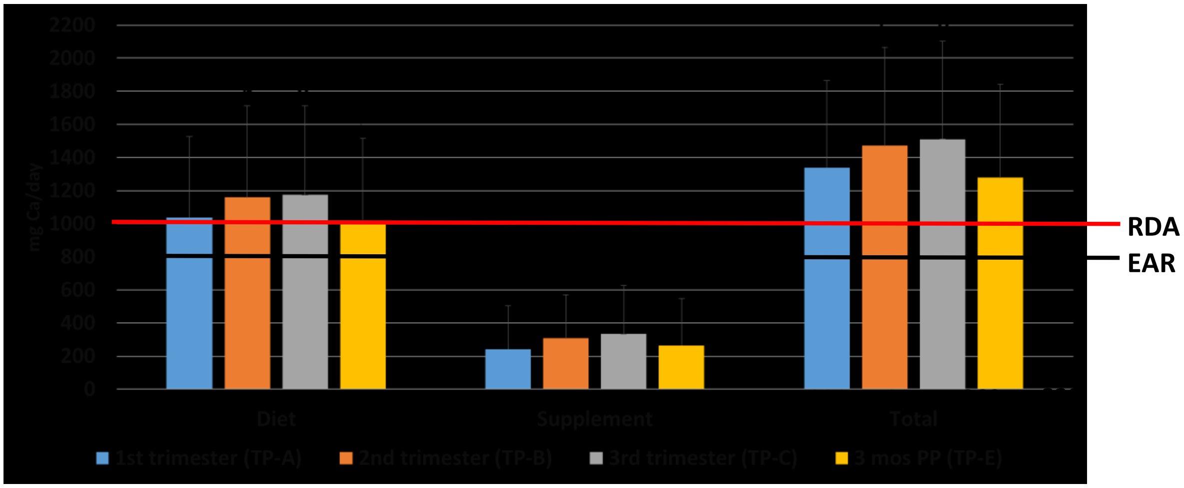


Figure 4.1: Estimated calcium intake during pregnancy and postpartum. Abbreviations: Ca-calcium, EAR- Estimated Average Requirement, mg- milligrams, RDA- Recommended Daily Allowance, and TP-time point. Bars represent mean ± standard deviation (SD). Means with different letters within a category of intake are significantly different (Two-sample Wilcoxon rank-sum test, $p < 0.05$).

Figure 4.2 illustrates mean estimated vitamin D intake during pregnancy and 3 months postpartum from diet, supplements, and total intake. Mean estimated dietary intake of vitamin D increased with each trimester, but was significantly lower at TP-E, compared to TP-B and TP-C. The highest proportion of women below the EAR for estimated total vitamin D was observed at TP-A (23%) and TP-E (23%); the highest proportion of women below the RDA occurred at TP-A (54%) and TP-B (48%). The proportion of women not meeting the EAR for total vitamin D was lower at TP-B (16%) and TP-C (13%). Only 1% of women at each time point were exceeding the UL. Vitamin D came predominately from supplements during pregnancy and 3 months postpartum (61-83% of total intake) in this cohort, and mean estimated dietary intake was below the EAR for all four time points.

Figure 4.2: Estimated vitamin D intake during pregnancy and postpartum

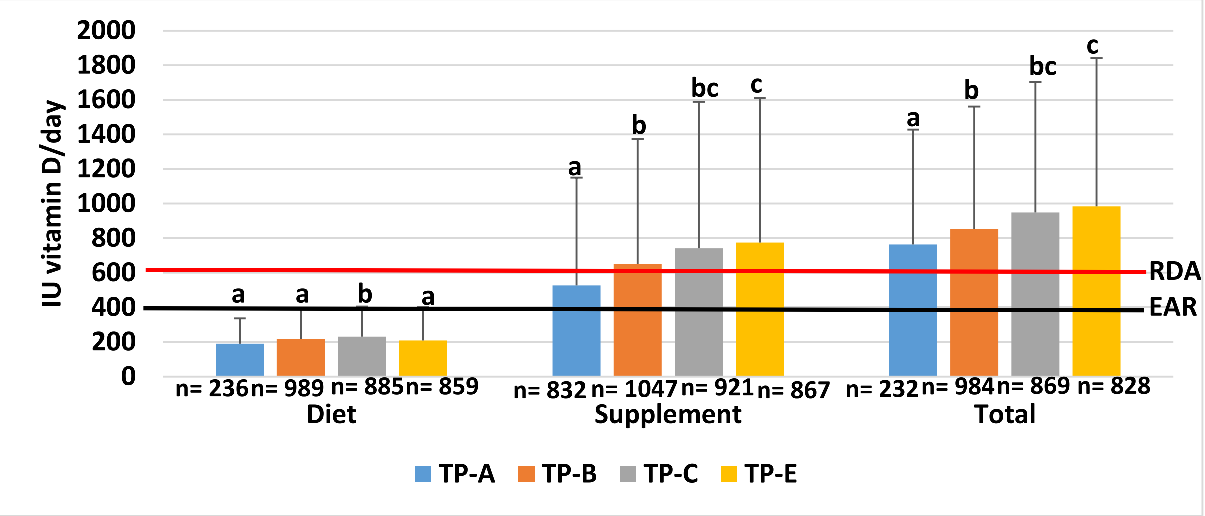


Figure 4.2: Estimated vitamin D intake during pregnancy and postpartum. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, RDA- Recommended Daily Allowance, and TP- time point, and. Bars represent mean ± standard deviation (SD). Means with different letters within a category of intake are significantly different (Two-sample Wilcoxon rank-sum test, $p < 0.05$).

4.2.1 Changes in estimated calcium and vitamin D intake over time

Figure 4.1a and **Figure 4.2a** shows estimated calcium intake and vitamin D intake, respectively, from participants that completed all four time points (n= 159). There were no significant differences in estimated calcium intake from diet or supplements during pregnancy and a significant decrease in intake from TP-C to TP-E **(Figure 4.1a)**. The significant differences in dietary vitamin D intake seen with the whole cohort did not exist in the repeated measures analysis; there were no significant differences in estimated diet intake for all four time points. With estimated supplement intake and total intake, there were no significant differences in vitamin D intake at TP-C when compared to TP-B and TP-E **(Figure 4.2a)**.

Figure 4.1a: Change in estimated calcium intake over time

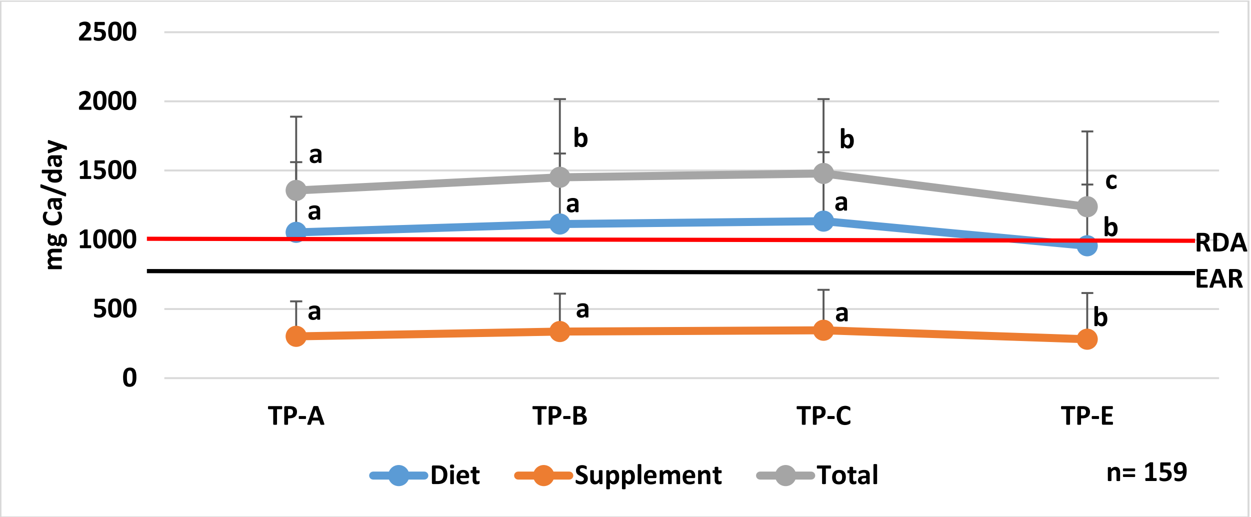


Figure 4.1a: Change in estimated calcium intake over time. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, mg- milligrams, RDA- Recommended Daily Allowance, and TP- time point. Lines represent mean ± standard deviation (SD) across all four time points. For each intake category, means with different letters are significantly different (Two-sample Wilcoxon rank-sum test, *p*< 0.05).

4.2a: Change in estimated vitamin D intake over time

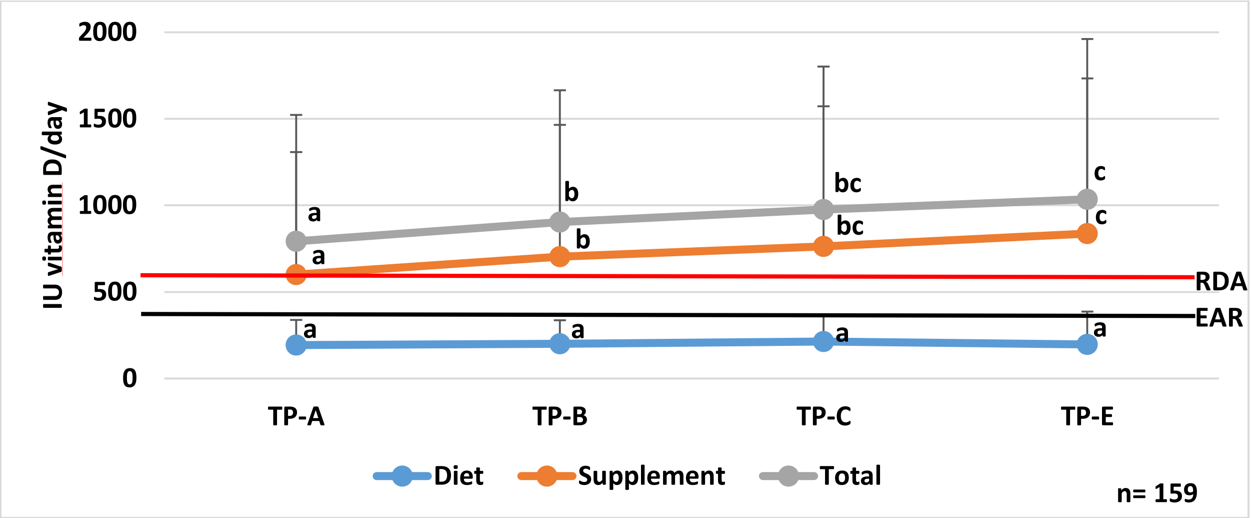


Figure 4.2a: Change in estimated vitamin D intake over time. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, RDA- Recommended Daily Allowance, and TP- time point. Lines represent mean ± standard deviation (SD) across all four time points. For each intake category, means with different letters are significantly different (Two-sample Wilcoxon rank-sum test, *p*< 0.05).

4.2.2 Estimated calcium and vitamin D intake based on milk drinking status

The estimated daily calcium intake from diet and total intake (diet and supplement) according to milk drinking status are seen in **Figure 4.3** and **Figure 4.4**. **Figure 4.5** and **Figure 4.6** shows the percentage of women meeting and not meeting the EAR based on milk drinking status by trimester for diet and total intake, respectively. Milk drinkers were categorized as such: no milk drinkers (0 mL/day), low (1 mL-250 mL/day), medium (250.1-500 mL/day), and high (> 500 mL/day). The highest proportion of those not meeting the EAR for both dietary and total estimated calcium occurred in women that drank no milk (55% and 57%, respectively) or low milk (41% and 40%, respectively). **Table 4.3 and 4.4** show the risk of not meeting calcium recommendations based on diet intake and total intake by milk drinking status. For diet intake, medium-high milk drinkers were 24.5 times (95% CI: 14.35-41.83, $p < 0.001$) more likely to meet the EAR than non-milk drinkers. Milk drinkers (any amount) were 4.8 times (95% CI: 3.02-7.78, $p < 0.001$) more likely to meet the EAR than non-milk drinkers in TP-C.

Figure 4.3: Estimated dietary calcium intake by milk drinking status

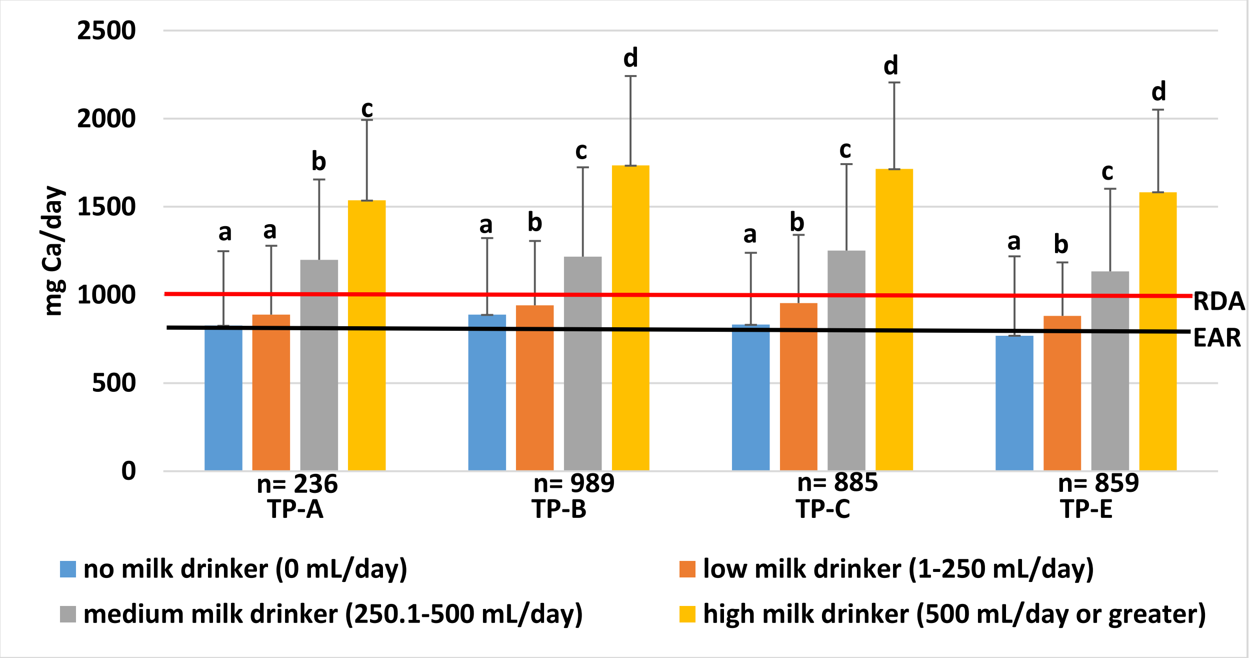


Figure 4.3: Estimated dietary calcium intake by milk drinking status. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, mg- milligrams, mL- milliliters, RDA- Recommended Daily Allowance, and TP- time point. Bars represent mean ± standard deviation (SD). Means within a time point with different letters are significantly different (Two-sample Wilcoxon rank-sum test, p< 0.05).

Figure 4.4: Estimated total calcium intake by milk drinking status

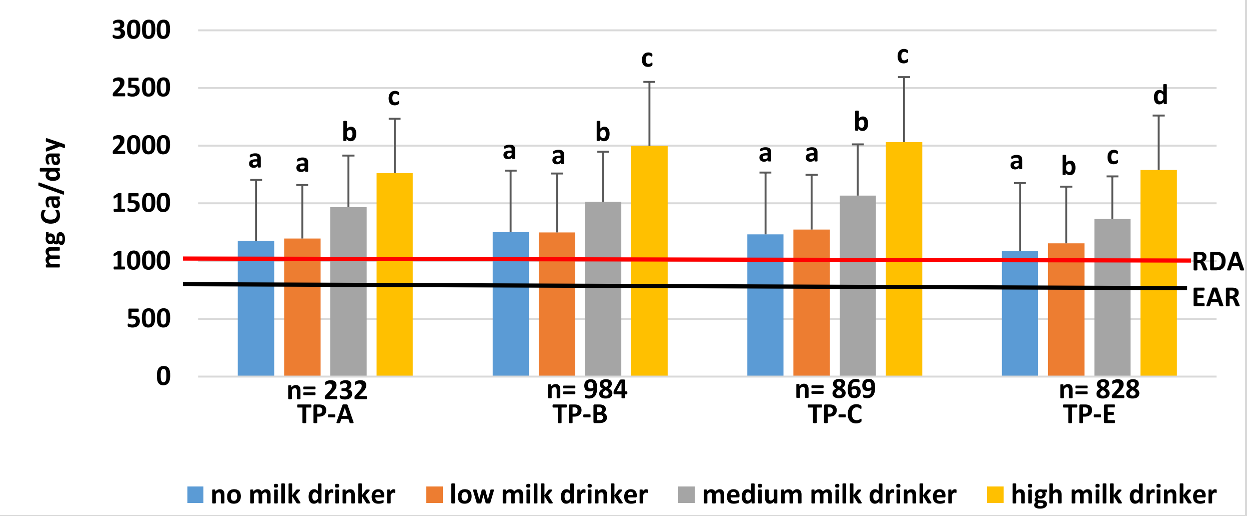


Figure 4.4: Estimated total calcium intake by milk drinking status. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, mg- milligrams, RDA- Recommended Daily Allowance, and TP- time point. Bars represent mean ± standard deviation (SD). Means within a time point with different letters are significantly different (Two-sample Wilcoxon rank-sum test, p< 0.05).

Figure 4.5: Percentage meeting the EAR for calcium (diet) by milk drinking status

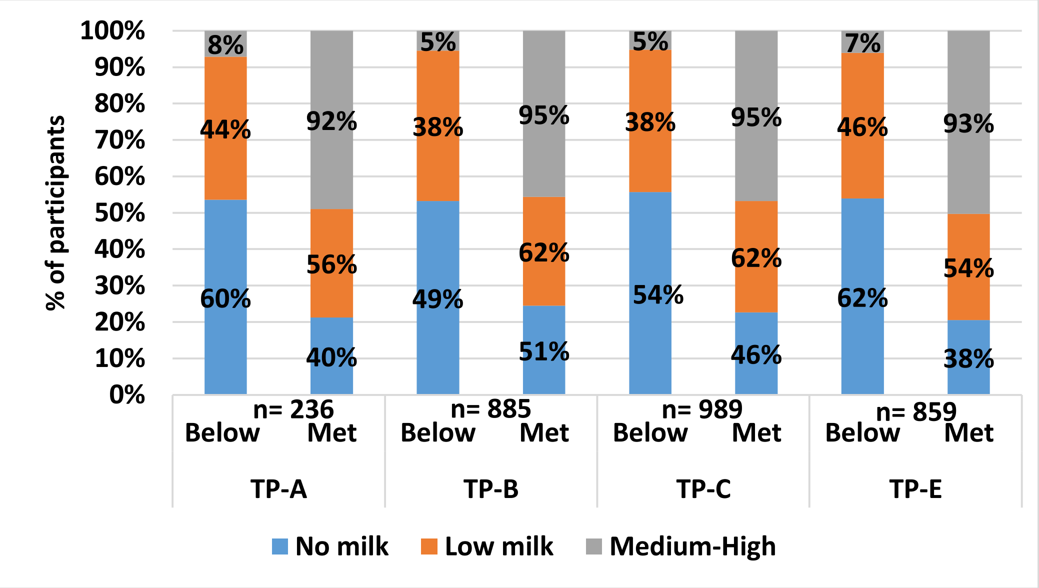


Figure 4.5: Percentage meeting the EAR for calcium (diet) by milk drinking status. Abbreviation: TP- time point. Medium and high milk drinkers were combined for analysis due to only a few women falling into these categories who were below the EAR.

Figure 4.6: Percentage meeting EAR for calcium (total intake) by milk drinking status

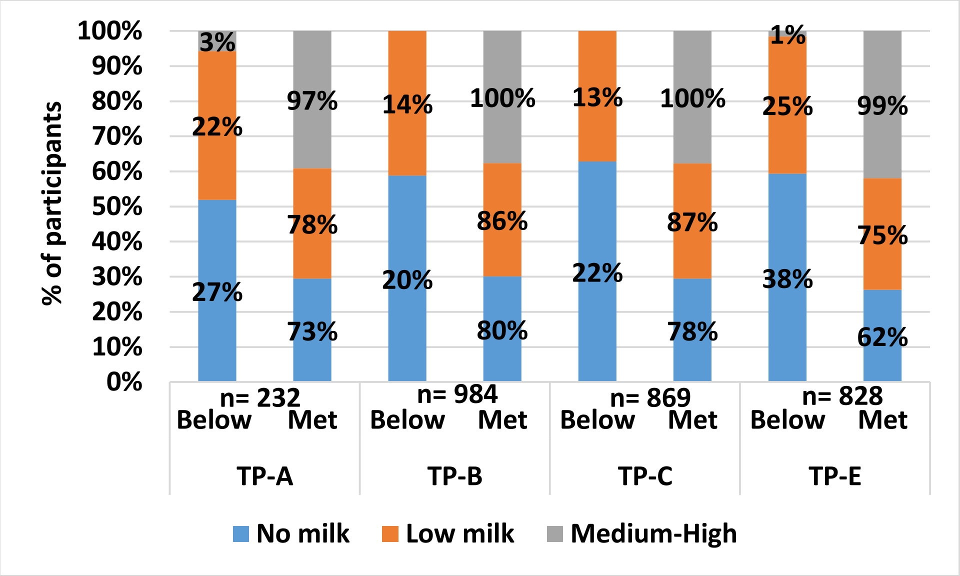


Figure 4.6: Percentage meeting the EAR for calcium (total intake) by milk drinking status. Abbreviation: TP- time point. Medium and high milk drinkers were combined for the analysis due to only a few women falling into these categories who were below the EAR.

Table 4.3: Odds ratio of meeting EAR for calcium based on milk drinking status (diet only)

Time point	**Milk drinking status**	**Odds ratio**	**95% CI**	***P* value**
TP-A	No milk	Reference		
	Low	1.9	(0.98-3.7)	0.056
	Medium-high	17.6	(7.24-42.82)	0.000*
TP-B	No milk	Reference		
	Low	1.6	(1.10-2.18)	0.012*
	Medium-high	16.9	(10.5-27.28)	0.000*
TP-C	No milk	Reference		
	Low	1.9	(1.3-2.73)	0.001*

	Medium-high	24.5	(14.35-41.83)	0.000*
TP-E	No milk	Reference		
	Low	1.8	(1.31-2.59)	0.000*
	Medium-high	22.1	(13.33-36.78)	0.000*

Odds ratio of meeting EAR for calcium based on milk drinking status for diet was determined using multinomial logistic regression. Abbreviations: CI- confidence interval and TP- time point. No milk drinker was used as the reference comparison. Medium and high milk drinkers were combined for analysis. A *p* value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

Table 4.4: Odds ratio of meeting EAR for calcium based on milk drinking status (total intake)

Time point	**Milk drinking status**	**Odds ratio**	**95% CI**	***P* value**
TP-A	No milk	Reference		
	Low, medium, high	2.9	(1.42-5.89)	0.003*
TP-B	No milk	Reference		
	Low, medium, high	4.3	(2.79-6.73)	0.000*
TP-C	No milk	Reference		
	Low, medium, high	4.8	(3.02-7.78)	0.000*
TP-E	No milk	Reference		
	Low, medium, high	4.4	(3.12-6.34)	0.000*

Odds ratio of meeting EAR for calcium based on milk drinking status for total intake was determined using multinomial logistic regression. Abbreviations: CI- confidence interval and TP- time point. No milk drinker was used as the reference comparison. All milk drinkers were combined for analysis. A *p* value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

Figure 4.7 (diet intake) and **Figure 4.8** (diet + supplements) shows estimated mean vitamin D intake per day in the cohort. There appears to be a dose effect that as milk consumption increases, estimated dietary vitamin D intake increases. There was no relationship between milk consumption and total estimated vitamin D intake. In fact, at TP-B and C, women that reported not drinking milk had a higher total estimated mean vitamin D intake than low milk drinkers likely due to compensation with supplements and fatty fish consumption, but this did not reach statistical significance. Only high drinkers were able to meet the EAR from TP-B onward with diet alone. When supplement intake was considered, all groups met the recommendations. **Figure 4.9** and **Figure 4.10** shows the proportion of women meeting and not meeting the EAR based on milk drinking status by trimester for diet and total intake, respectively, for vitamin D. Even among high milk consumers, approximately 78% of women were still not meeting the EAR with diet alone. When considering the contribution of supplements to total intake, approximately 53% of women with milk intake < 250 mL/day (no and low milk drinkers) did not meet the EAR. **Table 4.5 and 4.6** shows the likelihood of women meeting vitamin D recommendations based on milk drinking status from diet and total intake. At all four time points, women that drank milk were more likely to meet the EAR than those that did not drink milk, however this only reached significance for the medium-high milk drinkers **(Table 4.5)**. Even with factoring in supplement use (**Table 4.6**), still only medium-high milk drinkers were significantly more likely to meet recommendations.

Figure 4.7: Estimated dietary vitamin D intake by milk drinking status

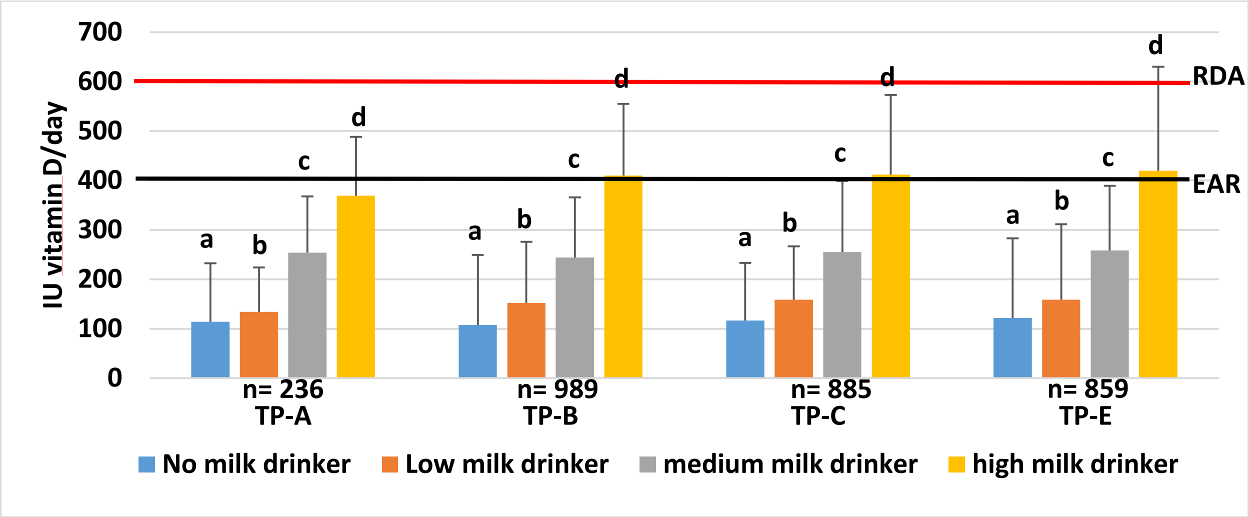


Figure 4.7: Estimated dietary vitamin D intake by milk drinking status. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, RDA- Recommended Daily Allowance, and TP- time point. Bars represent mean ± standard deviation (SD). Means with different letters within in a time point are significantly different (Two-sample Wilcoxon rank-sum test, *p*< 0.05).

Figure 4.8: Estimated total vitamin D intake by milk drinking status

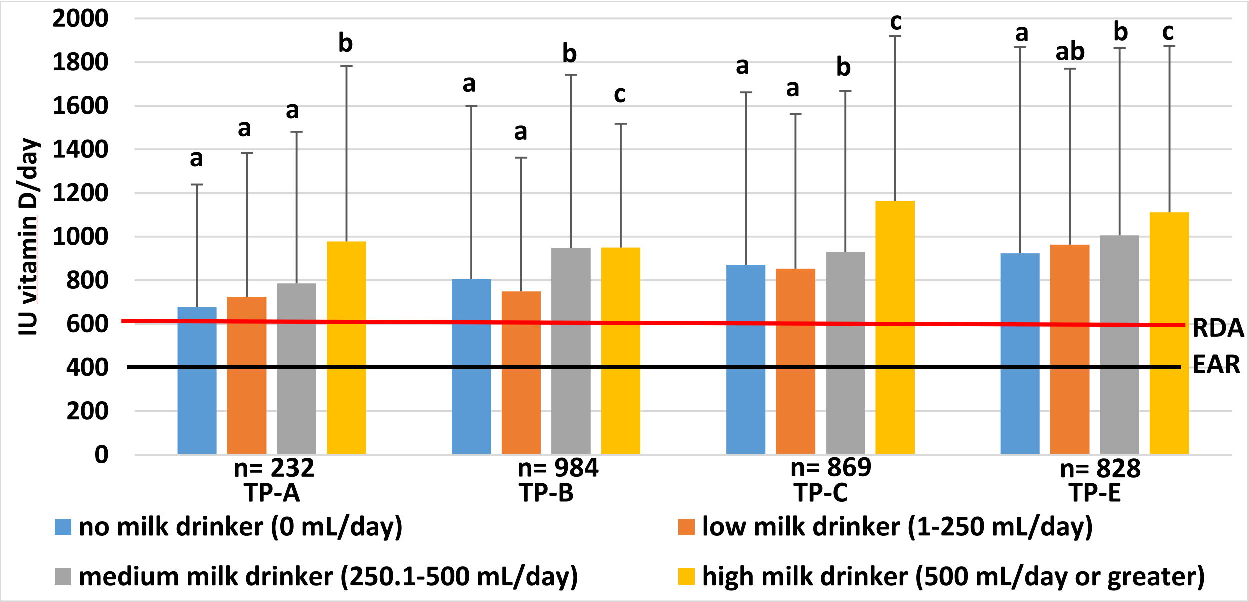


Figure 4.8: Estimated total vitamin D intake by milk drinking status. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, mL- milliliter, RDA- Recommended Daily Allowance, and TP- time point. Bars represent mean ± standard deviation (SD). Means with different letters within in a time point are significantly different (Two-sample Wilcoxon rank-sum test, *p*< 0.05).

Figure 4.9: Percentage meeting the EAR for vitamin D (diet) by milk drinking status

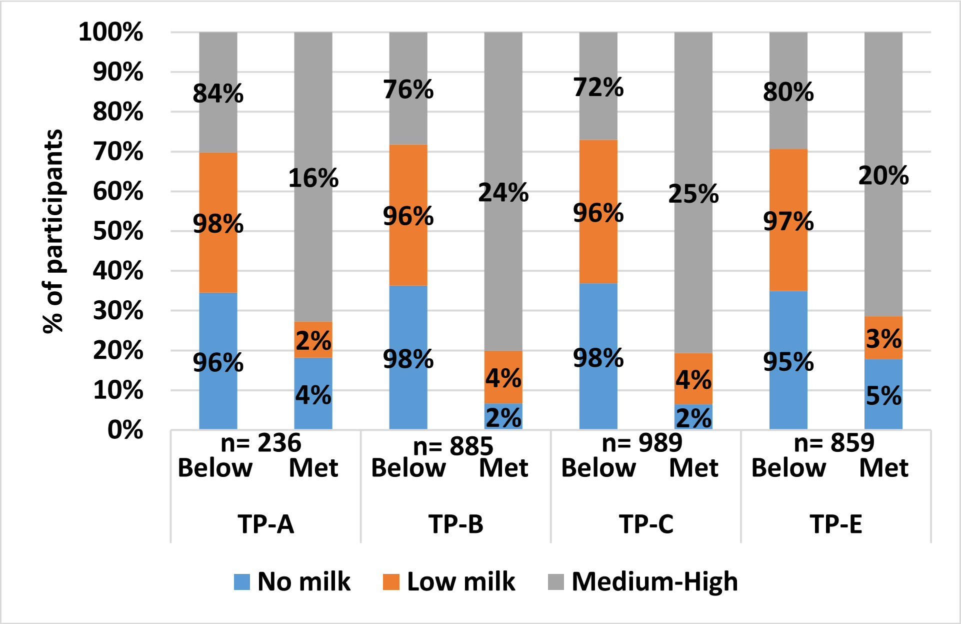


Figure 4.9: Percentage meeting the EAR for vitamin D (diet) by milk drinking status. Abbreviation: TP- time point. Medium and high milk drinkers were combined for the analysis due to only a few women meeting recommendations if they consumed no milk.

Figure 4.10: Percentage meeting the EAR for vitamin D (total intake) by milk drinking status

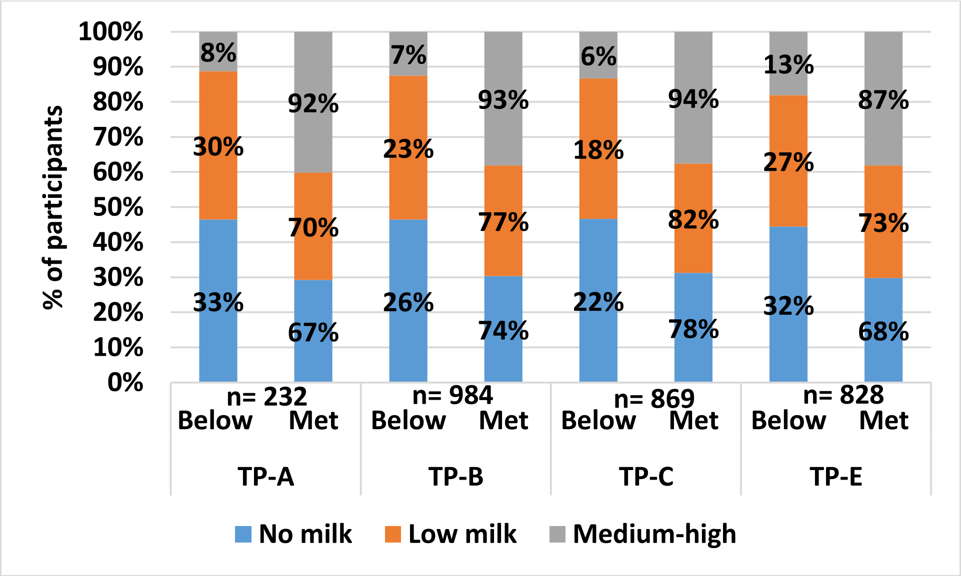


Figure 4.10: Percentage meeting the EAR for vitamin D (total intake) by milk drinking status. Abbreviation: TP- time point. Medium and high milk drinkers were combined for the analysis due to only a few women meeting recommendations if they consumed no milk.

Table 4.5: Odds ratio of meeting EAR for vitamin D based on milk drinking status (diet only)

Time point	**Milk drinking status**	**Odds ratio**	**95% CI**	***P* value**
TP-A	No milk	Reference		
	Low milk	0.4	(0.04-4.12)	0.455
	Medium-high	4.9	(1.34-17.56)	0.016*
TP-B	No milk	Reference		
	Low milk	2.1	(0.71-6.06)	0.181
	Medium-high	16.6	(6.66-41.2)	0.000*
TP-C	No milk	Reference		
	Low milk	1.6	(0.55-4.85)	0.379
	Medium-high	13.4	(5.39-33.56)	0.000*
TP-E	No milk	Reference		
	Low milk	0.7	(0.29-1.68)	0.418
	Medium-high	5.3	(2.81-10.13)	0.000*

Odds ratio of meeting EAR for vitamin D based on milk drinking status for (diet intake) was calculated using multinomial logistic regression. Abbreviations: CI- confidence interval and TP- time point. No milk drinker was used as the reference comparison. Medium to high milk drinkers were combined for analysis since few women who fell into these categories were below the EAR. A *p* value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

Table 4.6: Odd ratio of meeting EAR for vitamin D based on milk drinking status (total intake)

Time point	**Milk drinking status**	**Odds ratio**	**95% CI**	***P* value**
TP-A	No milk	Reference		
	Low milk	1.2	(0.57-2.37)	0.680
	Medium-high	5.7	(2.33-14.2)	0.000*
TP-B	No milk	Reference		

	Low milk	1.2	(0.80-1.76)	0.386
	Medium-high	5.1	(3.22-8.19)	0.000*
TP-C	No milk	Reference		
	Low milk	1.2	(0.78-1.96)	0.377
	Medium-high	4.3	(2.53-7.34)	0.000*
TP-E	No milk	Reference		
	Low milk	1.3	(0.87-1.84)	0.220
	Medium-high	3.2	(2.08-4.89)	0.000*

Odds ratio of meeting EAR for vitamin D based on milk drinking status for (total intake) was calculated using multinomial logistic regression. Abbreviations: CI- confidence interval and TP- time point. No milk drinker was used as the reference comparison. Medium to high milk drinkers were combined for analysis since few women who fell into these categories were below the EAR. A p value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

4.2.3 Estimated calcium and vitamin D intake related to macronutrient consumption

Table 4.7 reports estimated macronutrient and caloric intake (mean ± SD) of participants at each time point. The average caloric intake for the cohort was within the DRI guidelines for pregnancy and postpartum. Mean estimated daily protein and carbohydrate intake was higher, and fiber intake was lower than the DRI guidelines. Using a Kruskal-Wallis equality-of-populations rank test ($p < 0.05$), for all macronutrients, women meeting the EAR for calcium had significantly higher intakes than those not meeting the EAR **(not illustrated)**. Calcium intake was low to moderately correlated (0.35-0.50, $p < 0.001$) to caloric intake through pregnancy and postpartum **(Figure 4.11 and 4.12)**. There was no correlation between vitamin D intake and caloric intake (0.06-0.16, $p = 0.003\text{-}0.07$) (**Figure 4.13 and 4.14)**. When women were grouped into tertiles based on energy intake (low caloric consumption (500-1500 kcal), medium caloric consumption (1501-200 kcal), and (high caloric consumption (2001-4500 kcal)), estimated mean calcium intake significantly increased with each tertile except in TP-A where estimated calcium intake in the low caloric intake group was not significantly different from the medium caloric intake group **(Figure 4.15)**. When women consumed higher kcal/day, they had higher estimated calcium intake/day at all time points. While it may appear that the average intake of all three groups met the EAR for calcium during pregnancy and postpartum, a chi-squared test showed a higher proportion of women meeting the EAR for calcium in the high and medium calorie groups compared to the low calorie group at all four time points. For example at TP-B, 96% of women with high caloric intake, 91% with medium caloric consumption, and 60% with low caloric intake ($p < 0.001$) met the EAR**.**

Energy intake did not predict vitamin D intake since intake came mostly from supplement use. At TP-B and E, women that had high caloric consumption had lower estimated mean vitamin D intake than medium caloric intake **(Figure 4.16)**. A chi-squared test showed that 83% and 78% with high caloric intake at TP-B and E, respectively, met the EAR whereas 87% and 76% of women TP-B and E with medium caloric consumption met the EAR, respectively. Interestingly, at TP-C, women that had low caloric intake had higher mean vitamin D intake than those with medium intake caloric consumption. A chi-squared test showed 87% of women with low caloric intake met the EAR whereas 82% with medium caloric consumption met the EAR.

Table 4.7: Estimated mean (± SD) caloric and macronutrient intake across pregnancy and postpartum

Characteristics	**Results**	**DRI/guidelines for pregnancy/lactation**
Calories (kcal/day	2183 ± 621 (total)	1800-2350 kcal*
	TP A- 2069 ± 583	
	TP B- 2217 ± 621	

	TP C- 2259 ± 615	
	TP E- 2097 ± 625	
Protein (g/day)	91 ± 31 (total)	71 g/day** (both)
	TP A- 89 ± 32 (n= 236)	
	TP B- 91 ± 31 (n= 989)	
	TP C- 92 ± 32 (n= 884)	
	TP E- 89 ± 31 (n= 859)	
Fiber (g/day)	24 ± 10 (total)	28 g/day (pregnancy)** 29 g/day (lactation)**
	TP A- 22 ± 10 (n= 236)	
	TP B- 24 ± 11 (n= 989)	
	TP C- 24 ± 10 (n= 884)	
	TP E- 22 ± 10 (n= 859)	
Carbohydrate (g/day)	296 ± 94 (total)	175 g/day (pregnancy)** 210 g/day (lactation) **
	TP A- 279 ± 90 (n= 236)	
	TP B- 305 ± 94 (n= 989)	
	TP C- 311 ± 91 (n= 884)	
	TP E- 274 ± 92 (n= 859)	
Fat (g/day)	76 ± 32 (total)	No recommendation
	TP A- 72 ± 31 (n= 236)	
	TP B- 76 ± 32 (n= 989)	
	TP C- 77 ± 33 (n= 884)	
	TP E- 75 ± 33 (n= 859)	

Abbreviations: g- grams and TP- time point. Total represents mean ± standard deviation (SD) of all time points. * represents recommendations by Canada's Food Guide and ** represents recommendations by the IOM (Institute of Medicine).

Figure 4.11: Correlation of calcium intake to energy intake (all time points combined)

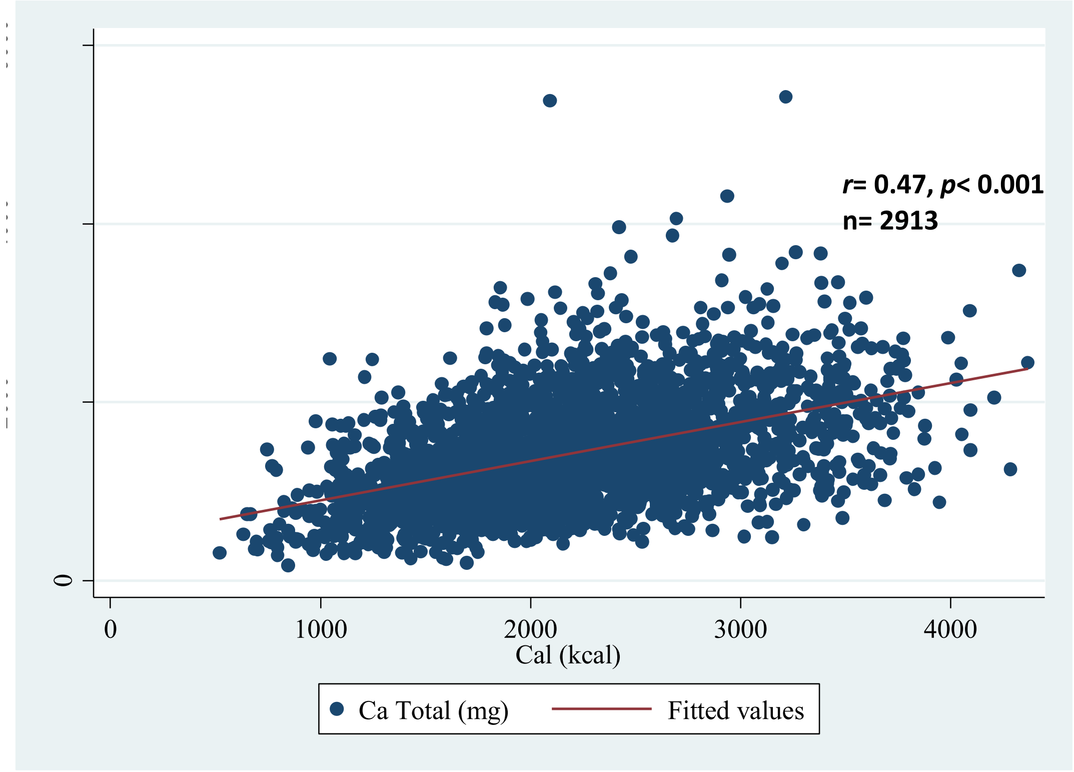


Figure 4.11: Correlation of calcium intake to energy intake (all time points combined). Abbreviations: Ca- calcium, kcal- kilocalories, and mg- milligrams. The x axis represents kilocalories/day and the y axis represents milligrams of calcium/day. The red line represents the line of best fit. A Spearman's Rank-Order Correlation of $p < 0.05$ was considered to be statistically significant.

Figure 4.12: Correlation of calcium intake to energy intake (by trimester)

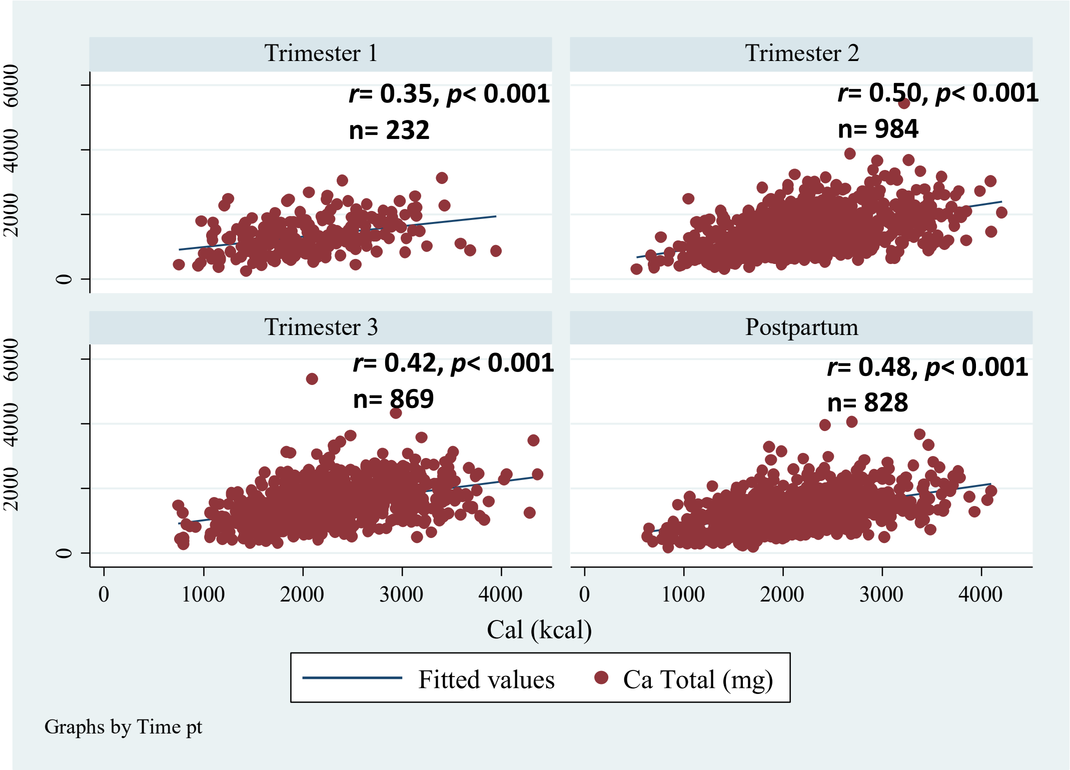


Figure 4.12: Correlation of calcium intake to energy intake (by trimester). Abbreviations: Ca- calcium, kcal- kilocalories, and mg- milligrams. The x axis represents kilocalories/day and the y axis represents milligrams of calcium/day. The blue line represents the line of best fit. A Spearman's Rank-Order Correlation with a $p < 0.05$ was considered to be statistically significant.

Figure 4.13: Correlation of vitamin D intake to energy intake (all time points combined)

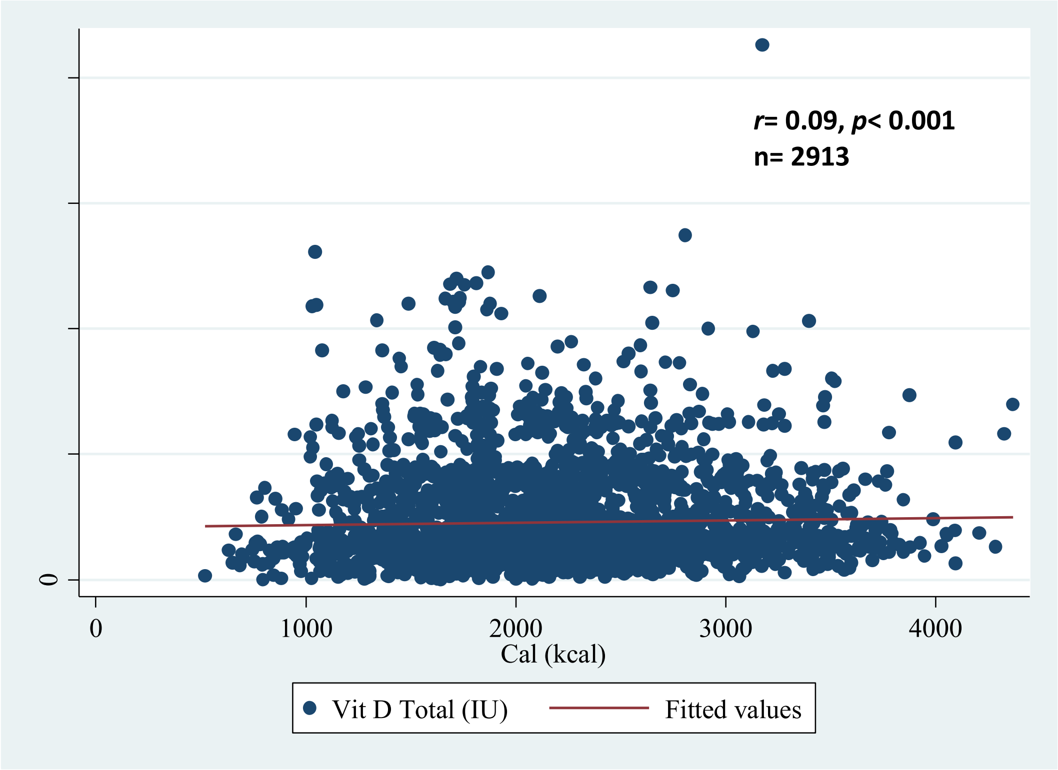


Figure 4.13: Correlation of vitamin D intake to energy intake (all time points combined). Abbreviations: IU- International Units, kcal- kilocalories, and Vit D- vitamin D. The x axis represents kilocalories/day and the y axis represents IU of vitamin D/day. The red line represents the line of best fit. A Spearman's Rank-Order Correlation of $p < 0.05$ was considered to be statistically significant.

Figure 4.14: Correlation of vitamin D intake to energy intake (by trimester)

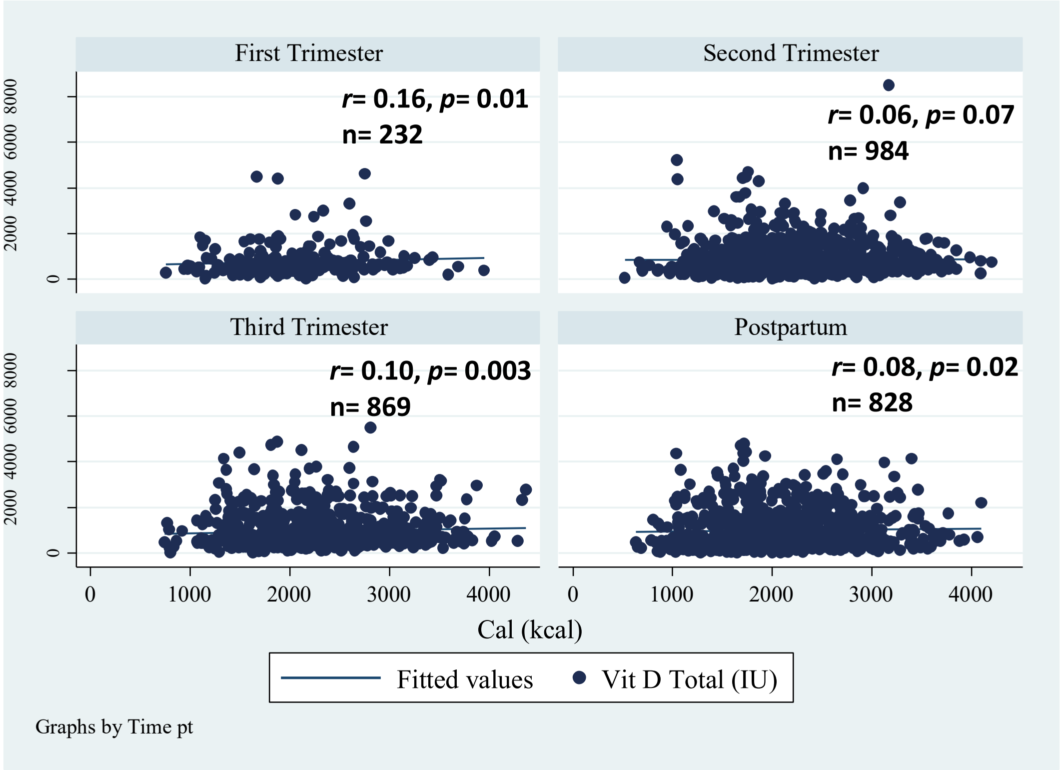


Figure 4.14: Correlation of vitamin D intake to energy intake (by trimester). Abbreviations: IU- International Units, kcal- kilocalories, and Vit D- vitamin D. The x axis represent kilocalories/day and the y axis represents IU of vitamin D/day. The blue line represents the line of best fit. A Spearman's Rank-Order Correlation of $p < 0.05$ was considered to be statistically significant.

Figure 4.15: Estimated mean total calcium intake by energy category

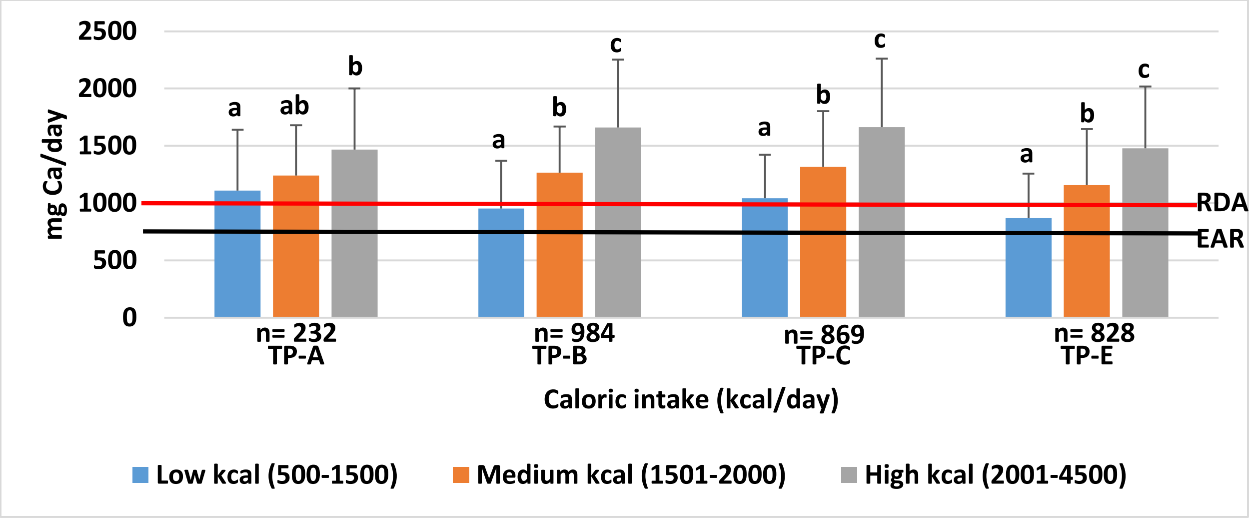


Figure 4.15: Estimated mean total calcium intake by energy category. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, kcal- kilocalories, mg- milligrams, and RDA- Recommended Daily Allowance. Bars represent mean ± standard deviation (SD). Means with different letters within a time point are significantly different (Kruskal-Wallis equality-of-populations rank test, $p < 0.05$).

Figure 4.16: Estimated mean total vitamin D intake by energy category

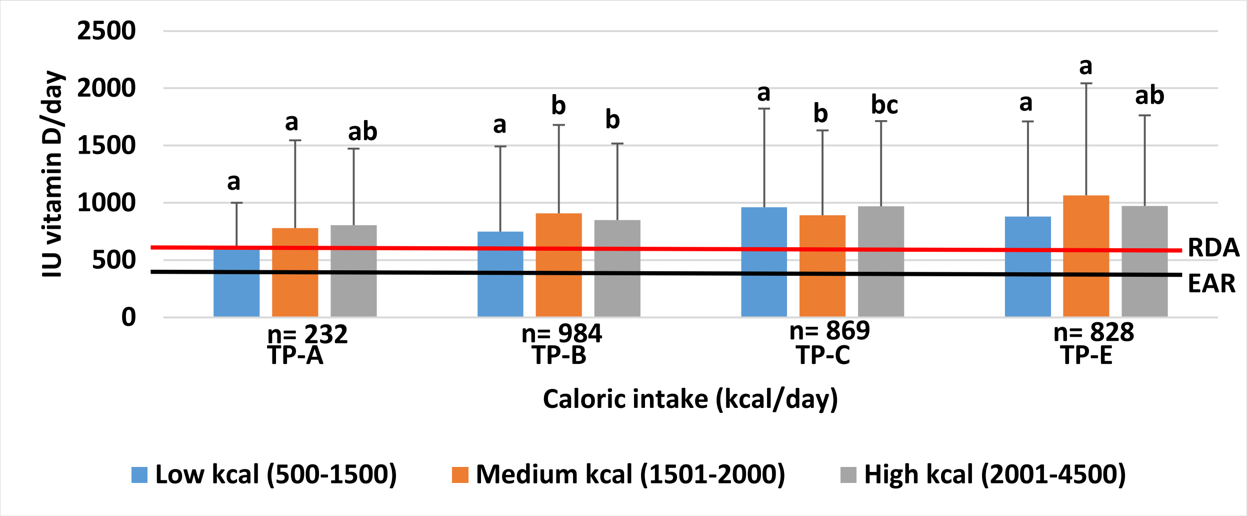


Figure 4.16: Estimated mean total vitamin D intake by energy category. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, kcal- kilocalories, RDA- Recommended Daily Allowance, and TP- time point. Bars represent mean ± standard deviation (SD). Means with different letters within a time point are significantly different (Kruskal-Wallis equality-of-populations rank test, $p < 0.05$).

Figure 4.17 and **Figure 4.18** expresses calcium and vitamin D intake/1000 kilocalories (kcal). When controlling for caloric intake, there were no significant changes in calcium intake across pregnancy but a significant decrease in mean estimated intake from TP-C to TP-E. For estimated vitamin D intake, there was an increase in estimated intake with each trimester and into TP-E. However, only the increase from TP-B to TP-C reached statistical significance.

Figure 4.17: Calcium intake expressed on an energy basis in the cohort

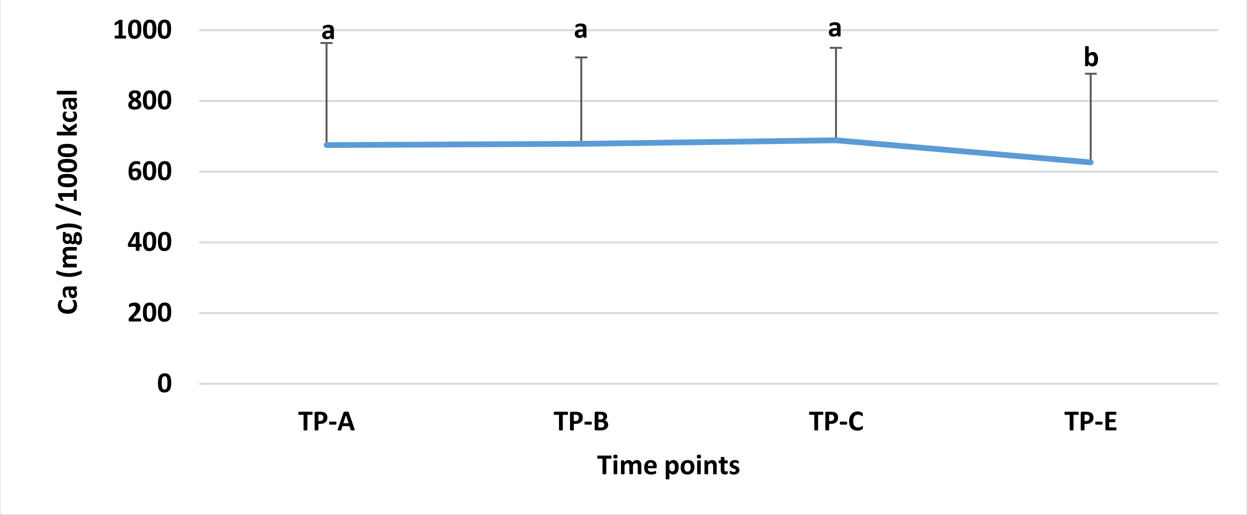


Figure 4.17: Calcium intake expressed on an energy basis in the cohort. Abbreviations: Ca- calcium, mg- milligrams, kcal- kilocalories, and TP- time points. Bars represent ± standard deviation (SD). At TP-A n= 232, TP-B n= 984, TP-C n= 869, and TP-E n= 828. Means with different letters are significantly different (Two-sample Wilcoxon rank-sum test, $p < 0.05$).

Figure 4.18: Vitamin D intake expressed on an energy basis in the cohort

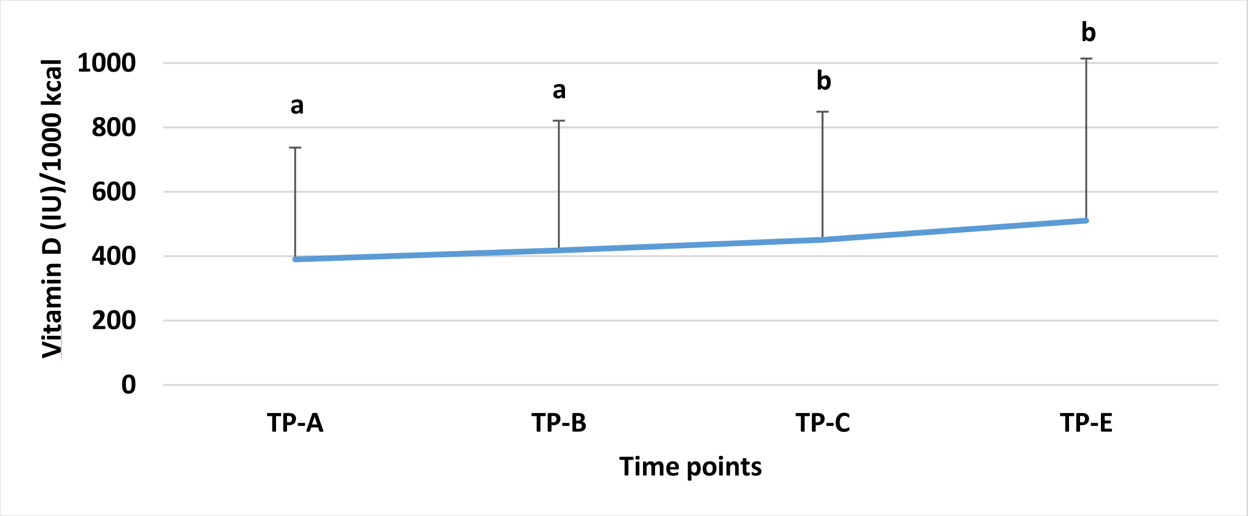


Figure 4.18: Vitamin D intake expressed on an energy basis in the cohort. Abbreviations: IU- International Units, kcal- kilocalories, and TP- time point. Bars represent ± standard deviation (SD). At TP-A n= 232, TP-B n= 984, TP-C n= 869, and TP-E n= 828. Means with different letters are significantly different (Two-sample Wilcoxon rank-sum test, $p < 0.05$).

4.2.4 Supplement users (SU) versus non-supplement users (NSU)

A greater proportion of women meeting the calcium recommendations were SU than NSU **(Figure 4.19)**. Approximately 33% of calcium NSU were not meeting the EAR and 87% of SU were meeting the EAR. As seen in **Figure 4.20**, both SU and NSU had estimated mean dietary intake above the EAR. There were no significant differences in dietary intake between SU and NSU across the time points (One-way ANOVA, $p > 0.05$). For TP-C and E, a significant difference in total intake was observed between SU and NSU (One-way ANOVA, $p < 0.05$). SU were 3.1 times more likely (95% CI: 2.18-4.33, $p < 0.001$) to meet the EAR than NSU. Approximately 84% of vitamin D SU met the EAR compared to 82% of NSU that were below the EAR **(Figure 4.21)**. Vitamin D SU were less likely (0.03) (95% CI: 0.02-0.05, $p < 0.001$) to not meet the EAR than SU. As seen in **Figure 4.22**, estimated mean intake of vitamin D from diet was below the EAR for both SU and NSU and there were no significant differences in mean estimated dietary intake between the two groups across all time points (One-way ANOVA, $p > 0.05$). However, there was a significant difference between estimated total intake for NSU and total intake for SU across all time points (Kruskal-Wallis equality-of-populations rank test, $p < 0.05$). In summary, a calcium containing supplement did not contribute substantially to meeting recommendations whereas a vitamin D containing supplements facilitated in better meeting recommendations.

Figure 4.19: Influence of a calcium containing supplement on meeting the calcium EAR

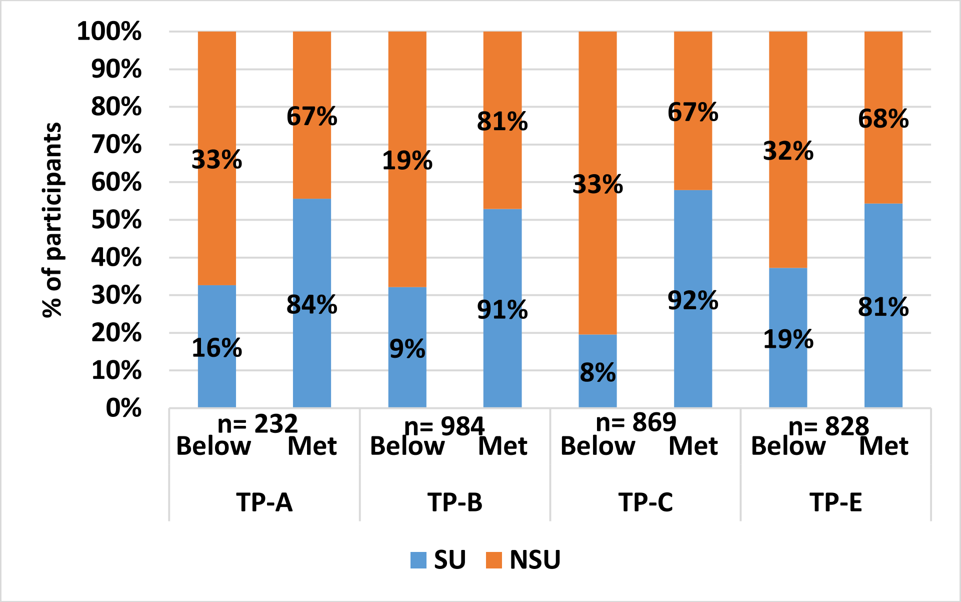


Figure 4.19: Influence of a calcium containing supplement on meeting the calcium EAR. Abbreviations: NSU- non-supplement user, SU- supplement user, and TP- time point. 'Below' indicates the percentage of women below the EAR and 'met' indicates the percentage meeting the EAR for calcium.

Figure 4.20: Mean estimated calcium intake based on supplement use status

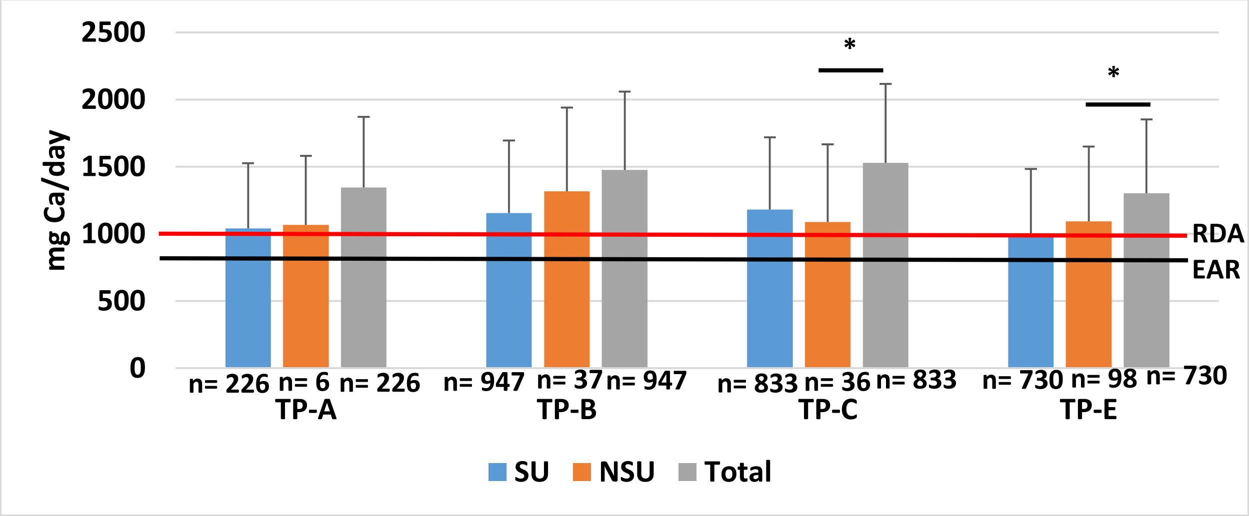


Figure 4.20: Mean estimated calcium intake (diet only) based on supplement use status. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, mg- milligram, NSU- non-supplement user, RDA- Recommended Daily Allowance, SU- supplement user, and TP- time point. Bars represent mean ± standard deviation (SD). The gray represents total estimated intake from diet as well as supplements from SU only. Only participants with both diet and supplement data could be considered for this analysis. The

orange represents total intake for NSU which is essentially only diet intake. The blue represents estimated intake from diet only of SU. * shows means between groups (total and NSU) that are statistically significant from one another (One-way ANOVA, $p < 0.05$).

Figure 4.21: Vitamin D supplement usage and meeting the EAR for total intake

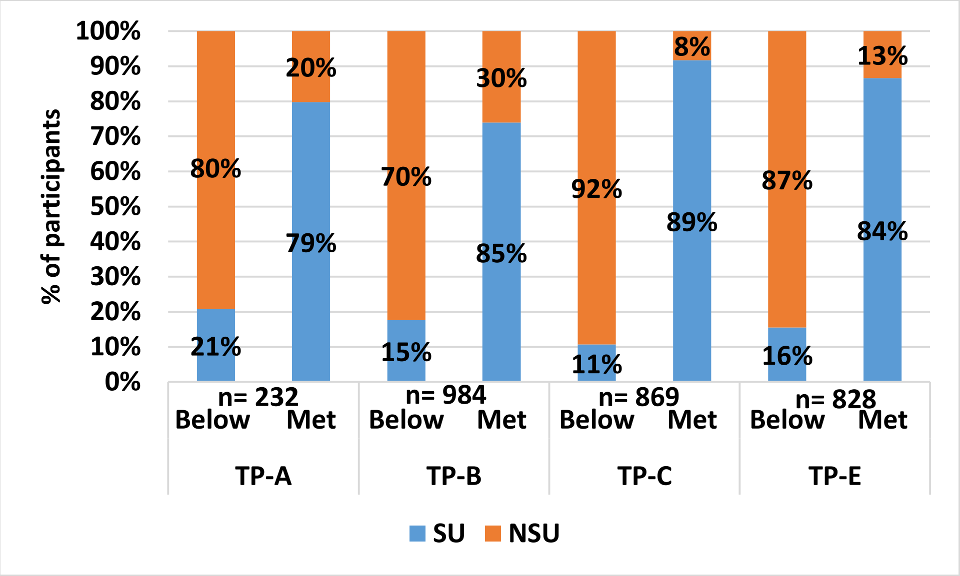


Figure 4.21: Vitamin D supplement usage and meeting the EAR for total intake. Abbreviation: SU- supplement user, NSU- non-supplement user, and TP- time point. 'Below' indicates the percentage of women below the EAR and 'met' indicates the proportion meeting the EAR for vitamin D.

Figure 4.22: Mean estimated vitamin D intake based on supplement use status

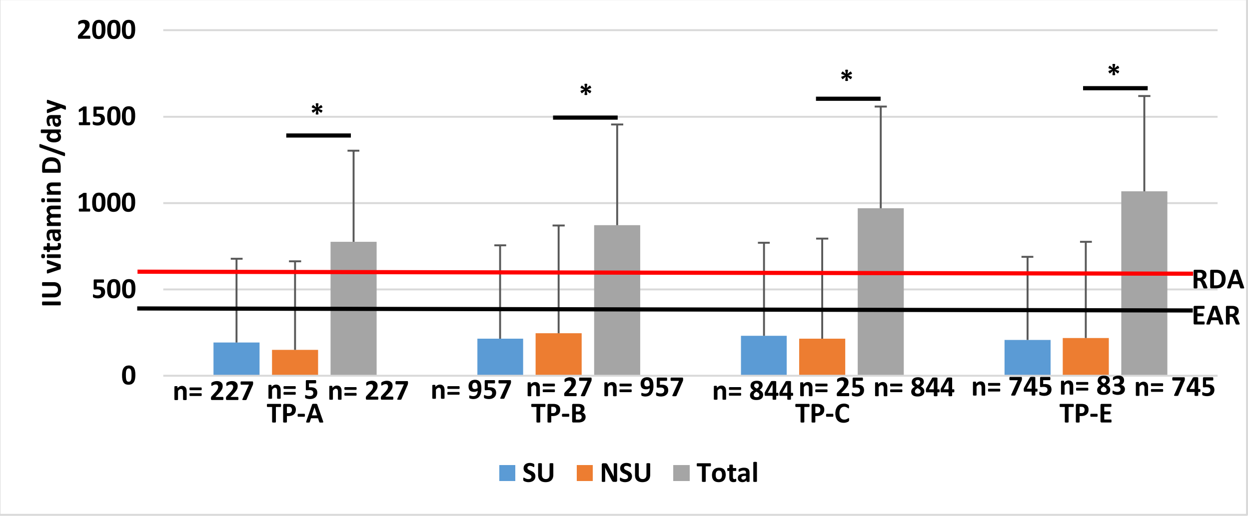


Figure 4.22: Mean estimated vitamin D intake (diet only) based on supplement use status. Abbreviations: EAR- Estimated Average Requirement, IU- International Units, NSU- non-supplement user, RDA- Recommended Daily Allowance, SU- supplement user, and TP- time points. Bars represent mean ± standard deviation (SD). The gray represents total estimated intake from diet as well as

supplements from SU only. Only participants with both diet and supplement data could be considered for this analysis. The orange represents total intake for NSU which is essentially only diet intake. The blue represents estimated intake from diet only of SU. * shows means the difference between total groups (orange and gray) are statistically significant from one another (Kruskal-Wallis equality-of-populations rank test, *p*< 0.05).

4.2.5 Calcium intake related to vitamin D intake

Women were categorized into four groups to determine risk for 'inadequate' calcium absorption and not meeting recommendations: those who fell below the EAR for both nutrients, those that met the EAR for calcium but not vitamin D, those that met the EAR for vitamin D but not for calcium, and those that met the EAR for both nutrients. Using this classification, nearly 1/3 of the APrON cohort was at risk for reduced calcium absorption or intake at each trimester of pregnancy and 34% were at risk during at TP-E **(Figure 4.23)**. Using multinomial logistic regression, women that were meeting the EAR for calcium were 5.8 times (95% CI: 4.64-7.38, *p*< 0.001) more likely to meet the EAR for vitamin D as well.

Figure 4.23: Total estimated calcium intake related to total estimated vitamin D intake by trimester

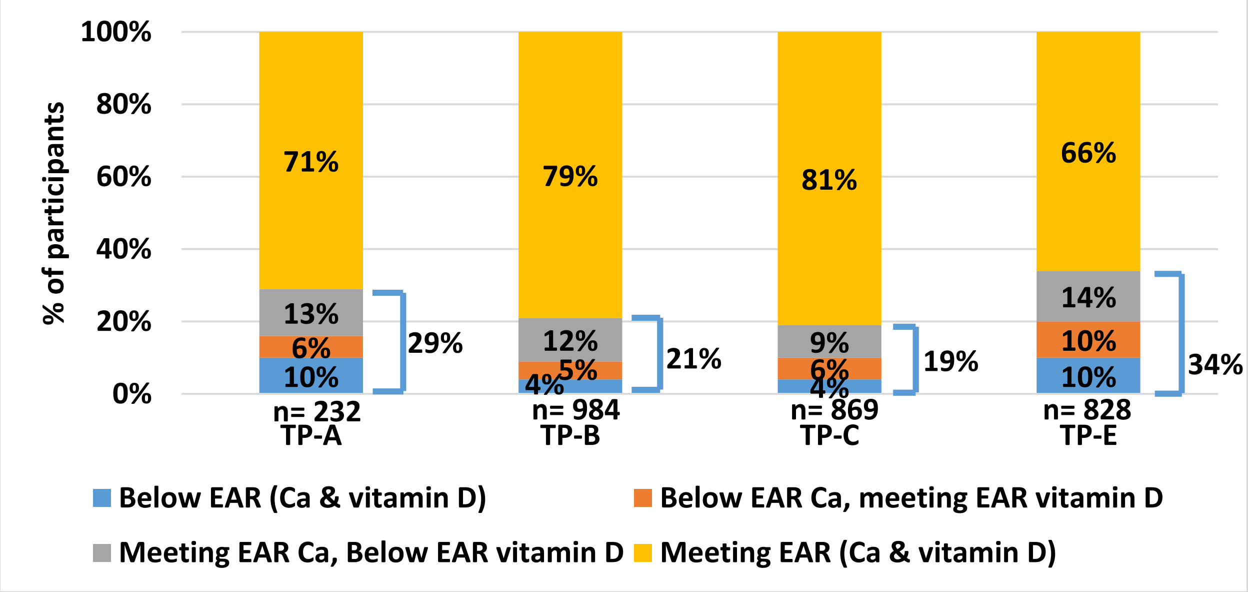


Figure 4.23: Total estimated calcium intake related to total estimated vitamin D intake by trimester. Abbreviations: Ca- calcium, EAR- Estimated Average Requirement, and TP- time point. Women were categorized in to four categories: those not meeting the EAR for Ca and vitamin D (blue), those below the EAR for Ca but meeting the EAR for vitamin D (dark orange), those meeting the EAR for calcium but below the EAR for vitamin D (grey), and those meeting the EAR for both Ca and vitamin D (light orange). The first three categories represent the women at risk for decreased calcium absorption and/or low intake.

4.3 Contribution of dairy and fortified foods and beverages to estimated calcium and vitamin D intake

It should be noted that cow's milk (whole, part-skimmed, skimmed, buttermilk, evaporated, and lactose-free) was considered for the analysis. A greater proportion of calcium from diet came from the fluid/powdered milk category (17-24%) than other dairy products such as cheese (7-12%) and yogurt (4-5%) during pregnancy and postpartum **(Figure 4.24)**. Calcium fortified cereal and other calcium fortified foods/beverages contributed to a lesser extent (4%) during TP-A and B than milk's contribution. At TP-E, the proportion of calcium coming from cereal and other calcium fortified foods returned to the proportions observed at TP-A and B (6%). Fortified foods and beverages were only a small proportion (3-6%) of total calcium in the diet of this cohort.

Figure 4.24: Contribution of dairy and fortified foods/beverages to estimated dietary calcium intake

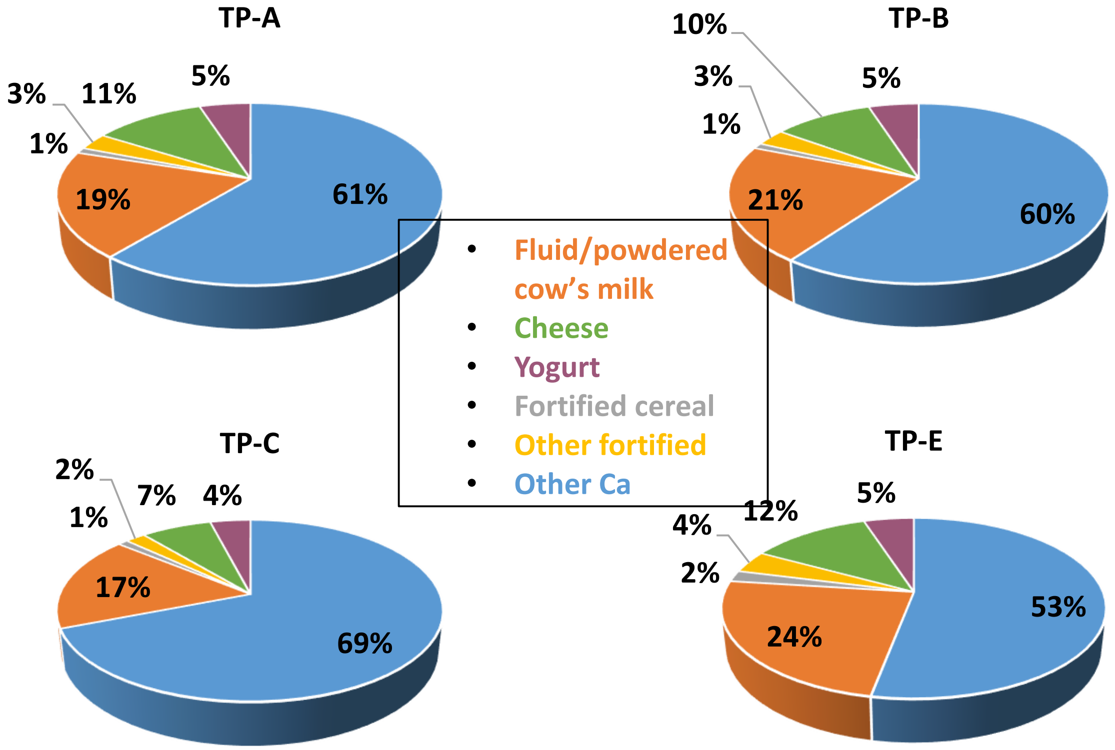


Figure 4.24: Contribution of dairy and fortified foods/beverages to estimated dietary calcium intake. Abbreviations: Ca- calcium and TP- time point. Each pie represents total estimated dietary calcium intake at each trimester of pregnancy and 3 months postpartum. Food/beverages were divided into six categories: fluid/powdered cow's milk (dark orange), cheese (green), yogurt (purple), fortified ready to eat cereals (grey), other fortified foods which included tofu, plant-based beverages, fortified orange juice, fortified flavored water, and fortified shakes (light orange), and other calcium (blue), which are other sources of calcium such as mixed dishes, vegetables, and fish.

Milk contributed 29-39% of vitamin D from diet during pregnancy and 40% during the TP-E **(Figure 4.25)**. Approximately 7% of total vitamin D from diet came from fortified food and beverage sources other than cow's milk. As with calcium intake, the greatest proportion of total vitamin D from fortified products other than cow's milk occurred at TP-E (9%). Fortified foods and beverages (excluding milk) made only a minor contribution to dietary sources of vitamin D.

Figure 4.25: Contribution of fortified foods/beverages to estimated dietary vitamin D intake

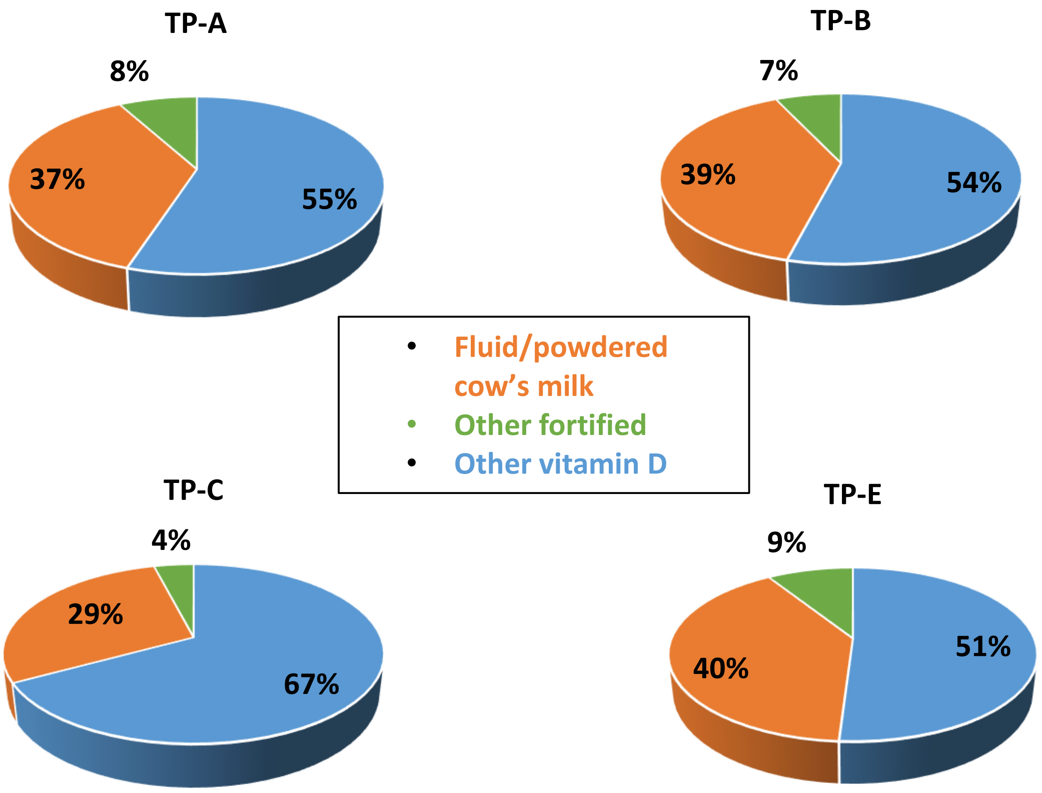


Figure 4.25: Contribution of fortified foods/beverages to estimated dietary vitamin D intake. Abbreviation: TP- time point. Each pie represents total estimated dietary vitamin D intake at each trimester of pregnancy and 3 months postpartum. Food/beverages were divided into three categories: fluid/powered cow's milk (orange), other fortified which included margarine, fortified orange juice, plant-based beverages, fortified flavored water, and fortified shakes (green), and other vitamin D sources in the diet such as eggs, mixed dishes, fish, beef, pork, and poultry (blue).

4.4 Co-variables in the statistical model and meeting recommendations

4.4.1 Co-variables for estimated calcium intake

Using Fisher's exact test, the following co-variables were found to significantly influence a woman's ability to meet the EAR for calcium during pregnancy: engaging in aerobic exercise (at TP-B only) (p= 0.016), whether the pregnancy was planned (p= 0.017), Caucasian race (p= 0.004), and desirable recommended weight gain during pregnancy (p= 0.008). **Table 4.8** shows the odds of women meeting the EAR with the characteristics that significantly influenced intake. Age, BMI, income, education, marriage status, parity, fertility treatment, smoking, and ethanol use had no significant influence on a women's ability to meet the EAR for calcium. Interestingly, women meeting gestation weight gain guidelines were 1.7 times (95% CI: 1.0-3.06, *p*= 0.05) more likely to meet the EAR for calcium than those that exceeded weight gain guidelines likely due to the inclusion of low fat dairy products **(Table 4.8)**. There was no significant difference in estimated mean total calcium intake between those with recommended weight gain (1414 ± 543 mg/day) and those below the recommended weight gain (1419 ± 658 mg/day).

Table 4.8: Odds ratio of meeting EAR for calcium by potential co-variables

Characteristic	**Odds ratio**	**95% CI**	***P* value**
Aerobic exerciser- during pregnancy (exerciser vs. non-exerciser)**	2.0 1.3 (all time points)	1.23-3.11 1.06-1.71	0.005* 0.016*
Planned pregnancy (planned vs. unplanned)	1.9	1.15-3.03	0.011*
Ethnicity (Caucasian vs. Non-Caucasian)	2.2	1.31-3.66	0.003*
Weight change during pregnancy (normal vs. excessive)***	1.7	1.0-3.06	0.05*

Odds ratio of meeting EAR for calcium by potential co-variables was determined using multinomial logistic regression. ** indicates measurement collected at TP-B and all four time points combined; aerobic exercise was found to not be statistically significant at any other time points. *** indicates measurement collected at TP-E. A P- value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

4.4.2 Co-variables for estimated vitamin D intake

Using Fisher's exact test, the following co-variables significantly influenced a woman's ability to meet the EAR for vitamin D during pregnancy: engaging in aerobic exercise (at TP-B only) (*p*= 0.001), marital status (married or common-law) (*p*= 0.046), and income (> $100,000/year) (*p*= 0.035). Age, smoking status, ethanol use, income, weight change status, planned pregnancy, BMI, education, parity, and use of fertility treatment had no significant influence on a woman's ability to meet the EAR for vitamin D. **Table 4.9** shows the odds of women meeting the EAR with the characteristics that significantly influenced intake. Women who were married/common-law were 4.2 times (95% CI: 1.11-15.83, *p*= 0.034) more likely to meet the EAR than divorced women. Women in the highest income bracket were 1.9 times (95% CI: 1.15-3.05, *p*= 0.034) more likely to meet the EAR than those in the middle income bracket.

Table 4.9: Odds ratio of meeting EAR for vitamin D by potential co-variables

Characteristic	**Odds ratio**	**95% CI**	***P* value**
Aerobic exerciser- during pregnancy (exerciser vs. non-exerciser)**	2.0	1.38-2.93	< 0.001*
Marital status (married/common	4.2	1.11-15.83	0.034*

law vs. divorced/separated)			
Income ($100K+ vs. $40-69.9K)	1.9	1.15-3.05	0.012*

Odds ratio of meeting EAR for vitamin D by potential co-variables was determined using multinomial logistic regression. ** indicates measurement collected at TP-B; aerobic exercise was found to not be statistically significant at any other time points. A *p* value of < 0.05 was considered to be statistically significant as indicated by an asterisk (*).

4.4.3 Infant Feeding Modality- Influence of Supplement Use and Meeting Recommendations

4.4.3.1 Calcium

At TP-E (n= 772), 68% of women exclusively breast fed, 23% combination formula and breast fed, and 9% exclusively formula fed. The greatest proportion of SU (70%) were women who exclusively breast fed, and 90% of women who exclusively breast fed reported taking a calcium containing supplement. Breast feeding and combination feeding women were 1.8 (95% CI: 0.92-3.62, p= 0.083) and 1.07 times (95% CI: 0.51-2.25, p= 0.856) more likely to take a calcium containing supplement, however both did not reach clinical significance. Seventy-one percent of women that exclusively breast fed met the EAR, and these women were 2.9 times (95% CI: 1.67-4.8, p< 0.001) more likely to meet the EAR for total estimated calcium than those that exclusively formula fed their infants. There was no significant relationship between milk drinking status and breast feeding status (Fisher's exact, p= 0.374).

4.4.3.2 Vitamin D

There was an association between breast feeding status and vitamin D supplement use (Fisher's exact, p= 0.004). Seventy percent of women that exclusively breast fed were 2.5 (95% CI: 1.22-4.93, p= 0.012) times more likely to take a vitamin D containing supplement than those that formula fed. There was an association between breast feeding status and meeting the EAR (Fisher's exact, $p < 0.001$). Eighty-one percent of women that exclusively breast fed met the EAR for vitamin D and these women were 2.3 times (95% CI: 1.37-4.0, $p = 0.002$) times more likely to meet the EAR for total vitamin D than those that exclusively formula fed their infants. Among women that combination fed and formula fed, 32% and 34%, respectively, did not meet the EAR. Breast feeding women that took vitamin D supplements were 2.2 times (95% CI: 1.17-4.23, $p = 0.014$) more likely to meet the EAR than those that formula fed.

Chapter 5: Discussion/conclusion

5.1 Discussion

5.1.1 Research objectives

The primary objective of this research was to describe calcium and vitamin D intake across the three trimesters of pregnancy as well as 3 months postpartum in a large cohort of women in Alberta (APrON). Several research questions were addressed to assess estimated intake at different time points, compare intake to the current DRI, identify women at risk for reduced calcium absorption, and identify predictor variables that would impact a women's ability to meet recommendations. The secondary objective was to identify the sources of calcium and vitamin D in the diet of the cohort. Several questions were addressed to assess macronutrient intake, compare intake from food groups, identify the contribution of fortified foods to overall intake, and assess milk drinking status and supplement use.

5.1.2 Intake of calcium and vitamin D in the APrON cohort

5.1.2.1 Estimated calcium and vitamin D intake at different time points

In the APrON cohort, estimated calcium intake came overwhelmingly from diet with minimal contribution from dietary supplements which was consistent with other studies reviewed (Harville et al., 2004; Rifas-Shiman et al., 2006; Mannion et al., 2007; Haugen et al., 2008). The estimated total calcium intake in the current study was consistent with Haugen et al. (2008) and Oken et al. (2007). Harville et al. (2004) was the only study that reported total estimated calcium intake (1671 mg) that was higher than the APrON cohort. The difference in results may be explained by the use of a FFQ which tended to over-estimate calcium intake (Block et al., 1992; Harville et al., 2004). Additionally, the group of women studied were WIC recipients who had received diet counseling and government food assistance which may have influenced food choices. Women who dropped out (15%) were not included in the study. Calcium supplement intake in APrON cohort constituted a similar proportion of estimated total intake (< 20%) as reported in the literature (Haugen et al., 2008). Estimated mean supplement intake in the cohort was similar to the average quantity found in a prenatal multivitamin confirming that these women did not take a separate calcium supplement.

Estimated calcium intake from diet, supplements, and total intake from the entire APrON cohort increased with each trimester of pregnancy however significantly decreased from TP-C to TP-E. This was the same trend as the repeated measures analysis which only included women that attended all four appointment visits (n= 159). Estimated mean dietary and supplement intake in TP-C was even higher (although not significantly) than first trimester intake when women are often unaware they are pregnant. The highest mean estimated intake of calcium from diet, supplements, as well as total intake in the cohort occurred at TP-B and TP-C of this cohort when symptoms of morning sickness such as nausea, emesis, and dysgeusia often dissipate and caloric intake subsequently increases as well. This corroborates with results of exclusively dietary intake from Derbyshire et al. (2009) and total intake from Rifas-Shiman et al. (2006). Estimated calcium intake from diet was also higher in this cohort than what has been reported for Canadian women of childbearing age (19-30 and 31-50 years old) which was 1102 ± 34 mg/day and 933 ± 34 mg/day, respectively (Statistics Canada*,* CCHS, 2004).

Our study is one of the first of its kind to examine estimated vitamin D intake from all sources (diet and supplements) during each trimester of pregnancy through to postpartum. In this cohort, pregnant and postpartum women had minimal vitamin D intake from diet and relied on vitamin D containing supplements to obtain vitamin D which was consistent with the literature (Rifas-Shiman et al., 2006; Haugen et al., 2008; Derbyshire et al., 2009; Viljakainen et al., 2010). Similar to Haugen et al. (2008), supplement intake in the APrON cohort made up greater than 50% of total estimated vitamin D intake.

Similar to calcium, estimated dietary intake of vitamin D in the entire APrON cohort as well as the repeated measures analysis (n= 159) increased with each trimester of pregnancy (although not significantly) but significantly decreased from TP-C to TP-E. However, in the repeated measures analysis, there were no significant differences in estimated intake from diet across all the time points which is consistent with Derbyshire et al. (2009). Interestingly, unlike calcium, reported vitamin D intake from supplements and total estimated intake at TP-E was the highest of all four time points. Vitamin D intake from supplements was approximately 6.25 µg-9.4 µg (250-375 IU) higher in the APrON cohort than reported in the literature (Rifas-Shiman et al., 2006; Oken at al., 2007; Haugen et al., 2008; Viljakainen et al., 2010). One might speculate that women in Calgary and Edmonton, Alberta are more aware of the need for supplemental vitamin D through promotion by health agencies and media outlets than in earlier studies (Caulfield et al., 2014).

5.1.2.2 Comparison to recommendations

In this cohort, pregnant and postpartum women met the EAR for calcium through diet, which is consistent with other studies (Harville et al., 2004; Rifas-Shiman et al., 2006; Haugen et al., 2008). However, Derbyshire et al. (2009) found that women were not meeting the EAR during pregnancy and postpartum with diet alone, but since supplement intake was not measured, comparisons based on estimated total intake could not be determined. One would expect dietary intake of these two studies to be similar since the baseline characteristics that were associated with higher calcium intake like being Caucasian, not smoking, and being older were similar (Harville et al., 2004; Rees et al., 2005; Haugen et al., 2008; Derbyshire et al., 2009; Sullivan et al., 2009; Rodríguez-Bernal et al., 2010). While there were other studies where women did not meet the EAR for calcium with diet alone, these studies estimated calcium intakes of women of child bearing age who were not currently pregnant (Hacker-Thompson et al., 2009; Bailey et al., 2010). Exceeding the UL for total estimated calcium was not an issue in our study (2-6% during pregnancy and 3% during postpartum) whereas Harville et al. (2004) had a greater proportion of women exceeding the UL (15%) due to a high use of calcium based antacids.

In this cohort, women had difficulty meeting the EAR for vitamin D from diet alone and relied heavily on supplements to meet recommendations. This is consistent with the literature which reported low intake from diet and a greater contribution to total estimated intake from supplements (Rifas-Shiman et al., 2006; Oken et al., 2007, Haugen et al., 2008; Derbyshire et al., 2009; Viljakainen et al., 2010). Only one study reviewed, reported pregnant women meeting the EAR with diet alone (12.4 µg (496 IU) ± 5.25 (210 IU)/day) (Oken et al., 2007). The differences in results may have been due to the FFQ used which was validated for use in the pregnant population but was not designed specifically for estimating vitamin D intake. While women in the APrON cohort met the EAR due to their respectable vitamin D containing supplement use, 13-23% of women during pregnancy and 23% during the postpartum period did not meet the EAR. It is important to identify that vulnerable subset of women with less than optimal intake. Exceeding the UL (1%) was not an issue in this population.

5.1.2.3 Relationship between estimated calcium and vitamin D intake

To the author’s knowledge, this is the first study to analyze estimated calcium intake in relation to vitamin D intake to identify those at risk due to low calcium intake and/or decreased absorption. By using only calcium intake, our study suggests that women are at low risk of not meeting current recommendations. However, sufficient vitamin D is required for absorption and metabolism of calcium, and when both vitamin D and calcium intake were considered, 19-29% of women during pregnancy and 34% at TP-E were at risk for decreased absorption as well as low intake. As high as 13% of women during pregnancy and 14% during the postpartum period were meeting the EAR for calcium but not for vitamin D. In addition, 6% during pregnancy and 10% during the postpartum period were not meeting the EAR for both nutrients. Given the high milk consumption of this cohort which is an excellent source of both calcium and fortified vitamin D, it was not anticipated that there would be a larger proportion of women who met the EAR for calcium but not vitamin D than those that met the EAR for both nutrients. This vulnerable subset may have been overlooked if only estimated calcium intake was assessed. These findings are particularly concerning to pregnant women who already experience normal bone loss during gestation and may be at risk for further loss as a result of decreased calcium absorption due to low vitamin D intake (Kalkwarf, 1999; Thomas and Weisman, 2006; Ross et al., 2011; reviewed by Hacker et al., 2012; Olausson et al., 2012). Furthermore, lactating women with decreased calcium absorption or intake are at risk for additional bone loss since they must meet their own body's needs in addition to the body sequestering calcium in breast milk for the suckling infant (Kalkwarf, 1999; Olausson et al., 2012). Additionally, 'sufficient' calcium intake as defined by 24-hour recall and supplement intake is predictive of 'sufficient' vitamin D intake. Women are more likely to meet the EAR for vitamin D if they are meeting the EAR for calcium. One would expect these results in the cohort given the large proportion of calcium intake from diet and the literature showing that vitamin D SU tend be to be older, have a lower BMI, and are well educated, which are characteristics descriptive of the APrON cohort (Haugen et al., 2008).

5.1.3 Predictor variables of intake

5.1.3.1 Breast feeding status

Since both calcium and vitamin D are sequestered in breast milk, it was imperative to identify if breast feeding women (exclusively or in combination with formula) were at risk for not meeting recommendations that could potentially impact infant outcomes. Women in the APrON cohort that exclusively breast fed had the highest proportion (84%) using a calcium containing supplement compared to women that exclusively formula fed or mixed fed their infant. However, women who exclusively breast fed their infants were not significantly more likely to consume a calcium containing supplement than women that formula fed. Women that exclusively formula fed had the lowest estimated mean dietary intake of calcium (860 ± 465 mg/day) as well as the lowest estimated calcium supplement intake (233 ± 239 mg/day) of the three groups at three months postpartum and were less likely to meet the EAR than women that exclusively breast fed. However, 29% of women that exclusively breast fed did not meet the EAR for calcium, illustrating that education/support is still needed in this group to ensure these women are meeting recommendations. Although women who combination fed (both breast fed and formula fed) were not significantly more likely to meet the EAR than formula feeders, they were identified as a vulnerable group as 28% did not meet the EAR for calcium. Women that combination feed and exclusively breast feed may be at risk for bone loss as the calcium needs of the breast fed infant takes priority over the mother's needs and their infants may also be at risk for not meeting the AI of 10 μg (400 IU)/day of vitamin D (Kalkwarf, 1999; Ross et al., 2011).

On the contrary, those that exclusively breast fed were significantly more likely to consume a vitamin D containing supplement as well as meet the EAR than women that formula fed. Still, 28% of women that exclusively breast fed did not meet the EAR for vitamin D. Even though 84% of formula feeders were vitamin D SU, 34% of them were still not meeting the EAR. Although combination feeders were not significantly more likely to meet the EAR than formula feeders, they are still a vulnerable group since 32% did not meet the EAR.

This is the first study known of its kind, in a healthy population, to analyze infant feeding practices in the first three months of life in regard to total intake, supplement use, and meeting the EAR. Previous research looked at women that exclusively breast fed (Mannion et al., 2007) as well as the overweight and obese population, and intake based on the three categories of infant feeding (Durham et al., 2011). Similar to our study, those that formula fed had a greater proportion of women not meeting vitamin D recommendations (Durham et al., 2011). Still, greater than 50% of women in each group were consuming less than 50% of calcium recommendations. The obese population is known to underreport intake greater than the general population which may explain the difference in our findings (Braam et al., 1998; Johansson et al., 2001; Durham et al., 2011). In summary, while breast feeding women are encouraged by healthcare professionals to supplement their infant with vitamin D daily, our research suggests that all Albertan women in the postpartum period should consider a vitamin D supplement to better meet current recommendations, and women who do not breast feed their infants should be encouraged to also take a calcium supplement to rebuild bone mass naturally lost as a result of pregnancy.

5.1.3.2 Caloric intake

Caloric intake influenced calcium and vitamin D intake differently in the cohort. Women with an estimated mean intake of < 1000 kcal/day did not meet the calcium recommendations but met the vitamin D recommendations at each time point except TP-A where it was slightly below the EAR. Consuming less than desirable calories during pregnancy and the postpartum period did not necessarily impact a women's ability to consume 'sufficient' vitamin D, however it did impact their ability to consume calcium. One would suspect that these results were due to calcium coming predominantly from diet and vitamin D intake coming overwhelmingly from supplements which is consistent with the literature. The results from our study contributes to the limited body of knowledge of calcium and vitamin D intake across pregnancy and into the postpartum period. Caloric density of calcium and vitamin D followed the same trends for estimated total calcium and vitamin D from the whole cohort. Estimated calcium intake increased with each trimester although not significantly and decreased significantly in the postpartum period. Estimated vitamin D intake increased with each trimester into postpartum however not significantly from TP-A to TP-B and TP-C to TP-E.

5.1.3.3 Other determinants of calcium and vitamin D intake

Besides supplement use and milk drinking status, aerobic exercise, planned pregnancy, ethnicity, and weight change status were identified as predictors of a woman's ability to meet the EAR for calcium in the APrON cohort. Other factors reported in the literature that were associated with significantly higher estimated calcium intakes but did not impact this cohort included older age, lower pre-pregnancy BMI, higher income, advanced education, being married, nulliparity, non-smoking status, and non-alcohol use (Harville et al., 2004; Haugen et al., 2008). Aerobic exercise, marital status, and income were found to significantly be associated with a woman's ability to meet the EAR for vitamin D in this cohort. Other factors reported in the literature that were found to be predictors of higher vitamin D status included alcohol use, increased age, ethnicity, lower pre-pregnancy BMI, advanced education, parity, and non-smoking status (Bodnar et al. 2007b). In the APrON cohort, we were more interested in whether or not these factors had an impact on meeting the recommendations since intake below recommended amounts are associated with adverse pregnancy and birth outcomes with both nutrients (reviewed by Hacker et al., 2012; reviewed by Olausson et al., 2012; Hypönnen et al., 2013). Prior studies have identified the proportion of women meeting recommendations, however this is the first study of its kind to examine the characteristics of the women not meeting recommendations. Only aerobic exercise during pregnancy, milk drinking, and supplement use were found to be predictors for both nutrients. Meeting calcium recommendations depended more on factors associated with leading a healthy lifestyle during pregnancy such as drinking milk, taking supplements, exercising, and gaining the recommended weight as defined by Health Canada, whereas vitamin D intake was associated more with socioeconomic factors such as marital status and income.

5.1.4 Sources of calcium and vitamin D in the APrON cohort

5.1.4.1 Food group comparisons

Milk had the greatest contribution to total estimated dietary calcium from Canada's Food Guide's, milk and alternatives group. The proportion of calcium intake from milk during pregnancy and postpartum was greater than other sources from this food group such as yogurt, cheese, and plant-based beverages. While milk and cheese were the two largest calcium contributors in our study, Harville et al. (2004) also reported breads and mixed dishes to be large contributors as well. While other sources of calcium were considered such as bread, mixed dishes, vegetables, and fish, the proportion of intake from each category was not determined for this thesis.

Besides vitamin D intake from eggs, fish, beef, pork, poultry, and mixed dishes which were included in the 'other vitamin D' category, milk had a sizeable contribution to total dietary intake. Whether one contributed greater to total estimated intake than the other, was not considered for this thesis. Since other dairy products are not good sources of vitamin D like milk which is fortified by law, they were not considered as well. A previous study had only estimated servings from various food groups in the first and second trimester without identifying the contribution to various micronutrient intakes (Rifas-Shiman et al., 2006). Our research extends the term of intake observation to include all of pregnancy and postpartum as well as the contribution of milk and other fortified sources of vitamin D.

5.1.4.2 Fortified food and beverage contribution

Fortified foods and beverages contributed very little (~4% during pregnancy and 6% during the postpartum period) to dietary calcium intake in the APrON cohort despite the rising popularity of plant-based beverages and fortified juices. Although milk consumption has decreased over time as reported in the literature, milk still made up the largest proportion of a fortified source of vitamin D in the diet of pregnant and postpartum women in the APrON cohort (Thomas and Weisman, 2006). Fortified dietary sources of vitamin D (including milk) were big contributors to intake in the APrON cohort, making up 33-36% of intake during pregnancy and almost 50% of consumption at 3 months postpartum. Our study extends the research on the contribution of various foods and food groups to dietary intake by including fortified sources of both nutrients which were uncommon to consume in North America approximately ten years ago and therefore were not coded for in the USDA Nutrient Database or Canadian Nutrient File used in nutrient intake analyses of the studies reviewed (Harville et al., 2004). One would expect that earlier studies estimated diet intake lower than the APrON study, however this was not always the case for calcium (Harville et al., 2004; Rifas-Shiman et al., 2006; Mannion et al., 2007). A more recent study by Fulgoni et al. (2011) that looked at naturally occurring and fortified/enriched sources of calcium and vitamin D in persons > 2 years old found a greater proportion of calcium as well as vitamin D from enriched/fortified sources than naturally occurring ones in the diet than our study. Our study is also the first to recognize the contribution of other fortified foods (such as margarine) and beverages (such as plant-based beverages, flavored water, and orange juice) to total intake and estimate the contribution fortified foods and beverages in the pregnant and postpartum population. This may explain such drastic differences in results.

5.1.4.3 Milk drinking status

This is the first study of its kind to analyze milk drinking status and its impact on dietary and total calcium and vitamin D intake during pregnancy; only one study reviewed, analyzed estimated intake during lactation. Women regardless of milk drinking status, met the EAR for calcium with diet alone for each trimester. It was only at TP-E that estimated mean intake for non-milk drinkers was found to be slightly below the EAR for calcium. This supports the literature showing that mean estimated calcium intake from diet was significantly higher for milk consumers than restrictors (Mannion et al., 2007). Prior research during the postpartum period showed mean intake above the EAR for calcium for ‘milk restrictors’ however, these were women consuming < 250 mL/day rather than not consuming any milk and all participants were exclusively breast feeding, which in our study has been shown to be associated with meeting the EAR (Mannion et al., 2007). As expected, drinking any quantity of milk (with the exception of the low milk intake in the first trimester) significantly increased the odds of meeting calcium recommendations. When considering estimated total calcium intake, our results support the literature that supplement intake facilitates milk restrictors to better meet recommendations (Mannion et al., 2007). Like calcium, women with limited milk consumption consumed on average less dietary vitamin D than their milk consuming counterparts which is consistent with the literature for the postpartum period (Mannion et al., 2007). Our results suggest that only high milk drinkers (> 500 mL/day) can meet the EAR with diet alone from TP-B onward. During TP-A, mean estimated intake of high milk drinkers failed to meet the EAR. Of the medium-high milk drinkers (> 250 mL/day), 84% of them did not meet the EAR. This is most concerning since the first trimester is a time of rapid cell proliferation and differentiation for the embryo and vitamin D plays a role in these processes (reviewed by Kaludjerovic and Vieth, 2010; Hypönnen, 2011). Since the APrON cohort women reported higher vitamin D supplement intake than the literature, total mean intake was substantially higher than other studies. Drinking a medium-high amount of milk (> 250 mL/day), significantly increased the odds of meeting vitamin D recommendations when considering diet intake and total intake. In fact, only 8% of women who were medium-high milk drinkers failed to meeting the EAR with the addition of supplements. In summary, estimated mean dietary intake of calcium and vitamin D differed significantly based on milk drinking status. Since 'inadequate' calcium intake will result in extraction from the bone, non-milk drinking women who plan to breast feed should be instructed to obtain additional servings of calcium rich foods/beverages or compensate with a calcium supplement. Our findings suggest that all pregnant and lactating women in Alberta regardless of milk drinking status, need to consume a vitamin D containing supplement to ensure they better meet the requirements of pregnancy and postpartum.

5.1.4.4 Calcium and vitamin D supplement usage

Haugen et al. (2008) investigated estimated dietary and total intake of calcium and vitamin D of SU and NSU, however our findings extend into the third trimester to 3 months postpartum. Based on the estimated mean supplement intake of SU in the APrON cohort, it is likely that calcium supplementation came from the use of a multivitamin and vitamin D from a combination of multivitamin and a separate vitamin D supplement. The overwhelming majority of APrON participants (96-98%) took a calcium or vitamin D containing supplement during pregnancy, however this decreased to 88-90% at TP-E. Taking a calcium or vitamin D containing supplement did not necessarily predict higher diet quality. In fact, SU did not have significantly different calcium or vitamin D dietary intake than NSU which is consistent with the findings from Haugen et al. (2008). Also, with the exception of TP-C, NSU had higher estimated mean calcium intake from diet than SU. Overall, calcium SU were still more likely to meet the EAR than NSU. In TP-B and TP-E, NSU had higher dietary vitamin D intake than SU. Overall, vitamin D SU were more likely to meet the EAR than NSU. Since our cohort had higher estimated vitamin D intake from supplements than reported in the literature, we had a higher proportion of women who were SU as well as NSU meeting the EAR than Haugen et al. (2008).

5.1.5 Limitations

A one day 24-hr recall was used to assess usual intake. The literature shows dietary intake can be more precisely estimated by increasing the number of dietary recall days and combining recall methods (Rush and Kristal, 1982; Subar et al., 2015). Self-reported data is not as accurate in measuring energy intake as other measures like DLW, for example, and will often under or over report intake (Dhurandhar et al., 2015; Subar et al., 2015). This would be problematic in estimating calcium intake accurately since there was a positive association between energy and calcium intake. Although the Canadian Nutrient File was used as the primary source for estimating nutrient intake, when foods or beverages could not be identified, the USDA database was used. The nutrient profile of a food may differ based on location as well as differences in fortification legislation. Lastly, this study's primary focus was to assess estimated intake during pregnancy and at 3 months postpartum and identify the groups of women at risk for not meeting nutritional recommendations, therefore, we cannot comment on nutritional status.

5.1.6 Future Direction

This study could be improved upon by incorporating the assessment of vitamin D status in predicting total estimated vitamin D intake across pregnancy as well as 3 months postpartum. It would also help to provide us a better understanding of women at risk for impaired calcium absorption. Our study only determined the risk of impaired bone health even though anthropometric measurements were taken during participants visits. A future study could assess estimated calcium and vitamin D intake in relation to outcome measures of birth and bone health in the mother and infant. Since participants also completed a FFQ along with the 24-hour recall in this study, a study combining both instruments used to estimate intake would strengthen the conclusions from our study. Lastly, a future study could extend the time period studied to include the 6 month postpartum follow up visit to enable comparison of estimated intake for mothers within the postpartum period.

5.2 Conclusion

In summary, in this high socioeconomic cohort, the majority of women were meeting the recommendations for calcium with diet alone but depended on supplement intake to meet vitamin D recommendations. Approximately 80% of women that did not consume a vitamin D containing supplement did not meet recommendations. Total estimated vitamin D intake increased during the postpartum period due to an increase in reported supplement intake while mean calcium intake declined from pregnancy to postpartum due to decreased supplement and dietary intake. Women that consumed milk had significantly higher odds of meeting calcium and vitamin D recommendations. When looking at both variables of intake together, a high proportion (33%) of women were at risk for decreased calcium absorption. Fortified foods and beverages, aside from milk, did not contribute substantially to total calcium intake from diet, however they provided a similar proportion of intake as yogurt. Consuming yogurt could facilitate in meeting calcium recommendations in women that for whatever reason do not consume milk. Since this study was limited to estimating intake and did not assess nutritional outcomes, an epidemiological study could follow the APrON women and look at the implications of low calcium and vitamin D intake on their status as well as the status of their infant.

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Appendix A: Calcium Content of Prenatal Multivitamins in Canada

Prenatal multivitamin	**Calcium content**
Centrum® Prenatal	250 mg
Nestlé® Materna®	250 mg
Pregvit®	300 mg
One a Day Women's Prenatal	400 mg
Prenatal (Exact/Kirkland brand)	200 mg
Nature Made Multi Prenatal	250 mg
Prenatal (Jamieson brand)	200 mg

Source: https://health-products.canada.ca/lnhpd-bdpsnh/index-eng.jsp