MILK VOLUME, MILK FAT AND CHILDHOOD FRACTURE

by

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Abstract

**Background:** Although cows' milk is believed to be an important dietary component for supporting bone health, limited research has addressed whether cows' milk consumption in childhood can reduce bone fracture risk.

**Methods:** This was a prospective cohort study of healthy urban children aged 1-10 years. Modified Poisson regression was used to test the association between milk volume and milk fat consumed between 1 and 3 years of age and risk of fracture between 3 and 10 years of age.

**Results:** Among the 2466 children, 153 sustained at least one bone fracture. Participants drank on average 1.9 cups of cows’ milk daily (2.5% average milk fat). Neither milk volume or milk fat consumed in early childhood were associated with fracture risk in later childhood.

**Conclusion:** Cows' milk consumption in early childhood was not protective against fracture in later childhood. These findings do not support increasing cows' milk consumption for fracture prevention.
Acknowledgements

I knew when I graduated from McMaster University that the U of T Masters in Nutritional Sciences was my next step. I had looked at many programs, and this master's degree was the only one I knew I wanted to apply to! I may not have known exactly what I wanted to research, but with the guidance and encouragement of Dr. Jonathon Maguire and my peers I found a research project that excited and challenged me. I have not only grown academically, but I have also gained practical skills. I have developed my critical thinking, independence, creativity and I could never have imagined how I would grow and learn over the last two years of this master's degree. I would like to thank Dr. Jonathon Maguire for his kind, encouraging and creative approach to research! I am thankful to have been part of such an innovative team working with TARGet kids! and all of our collaborators. Learning from such an accomplished and diverse group of professionals has pushed me beyond what I believed I was capable of. The drive all of the TARGet Kids! team members possess inspired and made me want to work harder to make a meaningful contribution to the research world.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>AA</td>
<td>Amino Acid</td>
</tr>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CCHS</td>
<td>Canadian Community Health Survey</td>
</tr>
<tr>
<td>CMPA</td>
<td>Cows’ Milk Protein Allergy</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CPS</td>
<td>Canadian Pediatric Society</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D Binding Protein</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intake</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>ECF</td>
<td>Extra Cellular Fluid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra Cellular Matrix</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin Like Growth Factor</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PHV</td>
<td>Peak Height Velocity</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RANK(L)</td>
<td>Receptor Activator of Nuclear factor-Kappa B – Ligand</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Control Trial</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Level</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>zBMI</td>
<td>z-score for Body Mass Index</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Childhood nutrition is important for optimal growth and development as well as disease prevention later in life (1). Bone fractures are common among healthy Canadian children and are on the rise (2). A child who has a bone fracture is at higher risk of subsequent fractures which can cause suffering and pose significant health care costs (2).

Cows' milk has been promoted as a good source of nutrients, such as calcium and vitamin D, to promote bone development in childhood (3-5). In Canada, cows' milk is fortified with Vitamin D and naturally contains Calcium (6). It is believed that both nutrients work synergistically to promote bone health especially during growth (7). However, there has been recent skepticism about the role that cows’ milk plays in childhood fracture prevention and cows’ milk consumption among children has been decreasing (8, 9). In North America, cows’ milk consumption by children has decreased by 7% from 1976-2006; during the same period, the percentage of children consuming whole milk has decreased from 60% to 32% (10). Similarly, CCHS 2015 data showed childhood milk consumption also decreases with age; 88% of Canadian children aged 1-3 had consumed milk on the previous day whereas 75% of children 4-8 years of age consumed milk on the previous day (11).

Pre-adolescent children are of particular interest within the context of cows' milk for fracture prevention, as fracture before puberty may be more sensitive to nutritional factors (12). Correlations between calcium, phosphorus and protein intake, and measures of bone strength appear to be strongest among pre-adolescent children (13, 14). In pre-adolescent children, bones are not fully mineralized yielding a scenario where fractures may be more sensitive to nutritional factors while in adolescents, fractures may be more sensitive to external factors such as vigorous physical activity and other high impact activities (12, 15, 16).

While associations between milk consumption and measures of bone turn over, bone mineral content (BMC), and bone mineral density (BMD) have been identified in observational studies (17, 18), there is much less evidence about the direct relationship between cows’ milk intake and fracture risk in children (17). Some have even suggested that current recommendations for childhood cows’ milk intake by Health Canada, the US National Institutes of Health and the American Academy of Pediatrics (3-5) may not be justified given the lack of evidence on the relationship between cows’ milk intake and fracture prevention (8, 19, 20).
The overall aim in this thesis is to evaluate the relationship between cows’ milk intake and childhood fracture risk. The thesis has been divided into the following sections:

Chapter 2 is a review of the literature about cows' milk and childhood fracture; Chapter 3 is my primary research titled: *Milk Volume, Milk Fat, and Childhood fracture*; Chapter 4 is an overall discussion of my results and how they fit into the existing literature; and Chapter 5 where I make overall conclusions, recommendations for future research and concluding remarks.
Chapter 2: Literature Review

1. Cows’ milk

Over the last 8000 years, milk from farmed cows has been a source of food for populations of European origin (9). Fortified cows' milk is an economical source of many nutrients which are challenging for children to receive from other dietary sources including, calcium, vitamin D, potassium and magnesium (21, 22). Cows’ milk contains approximately 87% water, 4.6% lactose, 3.4% protein, 4.2% fat, 0.8% minerals and 0.1% vitamins (23). The vitamin profile of cows’ milk consists of fat-soluble vitamins A, D and E and the water-soluble vitamins B and C (21, 24). Cows’ milk macronutrient content is similar to human breast milk which is regarded as the best source of nutrition for children in the first year of life (25). The macronutrient distribution of cows’ milk includes 50% carbohydrates, 30% fats, and 20% proteins (1). Although cheese and yogurt are fortified with vitamin D the serving size recommended does not provide adequate levels of vitamin D for optimal bone growth, making fortified cows' milk, the preferred dairy source (17, 26). Fortified cows’ milk has been shown to improve biochemical indexes of bone acquisition more than calcium supplementation alone (17, 18). Since the early 1960’s, cows’ milk has been available to consumers in a variety of fat percentages (27) (Figure 1 (1)).
Figure 1 – Nutritional Content of Cow Milk (1, 28)

1.1 Dietary Recommendations

At 1 year of age the National Institutes of Health and the American Academy of Pediatrics recommend starting children on whole cows’ milk (3-5), whereas Health Canada recommends this between 9-12 months (29). Health Canada recommends not starting children on cows’ milk before 9 months of age in efforts to reduce the risk of iron deficiency (30). Whole cows’ milk is recommended for children younger than 2 years of age because calorie needs are high and dietary fat is important for brain development (3-5). Health Canada's "Eating well with Canada's Food Guide" recommends that children 2-8 years of age consume 2 servings of reduced fat milk/dairy or dairy alternatives including cheese, fortified soy beverage, yogurt and kefir (5) (Table 1). Recommendations around lower fat dairy and alternatives for children older than 2
years of age are intended to reduce the risk of excessive weight gain and health problems related to excess adiposity later in life (5, 31).

Table 1 – Early Childhood Cows’ Milk Recommendations based on Organization

<table>
<thead>
<tr>
<th>Organization</th>
<th>Age of Introduction</th>
<th>Milk fat Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Canada (5, 29)</td>
<td>9-12 Months</td>
<td>Whole Milk until 2 years of age. After 2, introduce lower fat milk and alternatives.</td>
</tr>
<tr>
<td>National Institutes of Health (7)</td>
<td>12 months</td>
<td>Children younger than 2 years of age should only receive 3.25% cows’ milk. Over 2 years of age should switch to 1% or 2% cows’ milk.</td>
</tr>
<tr>
<td>American Academy of Pediatrics (4)</td>
<td>12 Months</td>
<td>Children younger than 2 years of age should only receive 3.25% cows’ milk</td>
</tr>
<tr>
<td>Canadian Pediatrics Society (32)</td>
<td>9-12 Months</td>
<td>Between 2-5 years of age 1%, 2% and 3.25% cows’ milk is recommended. After 5 years of age skim milk may be introduced</td>
</tr>
</tbody>
</table>

1.2 Legislated Requirements

1.2.1 Milk Processing

To provide the safest and highest quality beverage to consumers, cows' milk in Canada has been legislated to be processed in a standardized way since the early 1900’s (33). Under sanitary conditions, milk is harvested, cooled and stored at 7°C, a maximum 2 hours from time of milking. The milk is transported to the processing plant where it is tested, pasteurized and fractioned into various milk fat concentrations. First, the milk is divided into two groups via
centrifuge, skim milk (0.01% fat) or cream (40% fat). Then, milk fat is partially added back to create the varieties of milk familiar to the consumer. Milk is subsequently pasteurized for two primary purposes, to increase the safety for the consumer by eliminating disease-causing microorganisms and to increase the keeping quality of milk by removing spoilage microorganisms (34).

1.2.2 Fortification

Cows' milk contains naturally occurring calcium, however nutrients that do not occur naturally or are stripped during processing must be added. Fluid milk in Canada is legislated to contain vitamin A and vitamin D according to Health Canada's Food and Drugs Act. To achieve legislative fortification requirements vitamins are prepared as per regulatory requirements in the form of dry premix or fluid vitamin solution and added to milk under strict quality assurance standards. Vitamin D is added to all forms of cow's milk whereas vitamin A is only added to the skimmed milk varieties (35). Vitamin A is abundant in animal milk (14), however, during the separation process of the skimmed varieties of milk, vitamin A, which is fat soluble, is removed. Vitamin A remains in Homogenized (whole) milk, and fortification is not necessary. Once fortification is complete, the milk is pasteurized, packaged and distributed to retailers (35).

1.3 Nutrient Composition

1.3.1 Calcium

Cows' milk is a convenient source of dietary calcium and one of the most abundant sources of calcium per calorie (1, 9). The recommended dietary allowance (RDA) of calcium for children 1-3 years of age is 700mg/day (36). One 250mL cup of cows' milk contains approximately 250mg of calcium (37). Therefore, two cups of cows’ milk provide children with 60% of the calcium RDA (38). Children who do not consume cows’ milk tend to have dietary calcium intakes below the RDA (17, 39). Inadequate calcium intake during growth may increase the risk of fracture during childhood and predispose children to lower peak bone mass (17, 19). Further, variations in calcium intakes during childhood may account for a 10-15% difference in peak adult bone mass (13).

Calcium intake is known to influence bone mass and is important in childhood for optimal bone development (9). Calcium is necessary for many biological pathways including
neurotransmitter release, muscle contraction, and blood coagulation (1). Dietary calcium is absorbed either through active (vitamin D dependent) or passive transport across the intestinal mucosa (7, 9). Active transport occurs primarily in the duodenum where the vitamin D receptor (VDR) is expressed in its highest concentration (7). During low and moderate calcium intake active transport is stimulated by calcitriol, the biologically active form of vitamin D. Passive transport is dependent on luminal:serosal electrochemical gradients and occurs during high calcium intake states (7). Passive diffusion can occur over the length of the intestine however, the highest diffusion occurs in the duodenum and jejunum (7, 40). Fractional calcium absorption is defined as the ratio between dietary calcium utilized and calcium consumed (41) and is calculated by the US Institute of Medicine (IOM) using the factorial method. Fractional calcium absorption is about 25% for adults but in growing children with high calcium needs, fractional calcium absorption averages ~45% for children 1-3 years of age (7). From Lynch and colleagues, in children aged 1-3 years the average calcium accretion is 142mg/day, urinary loses are 34mg/day and endogenous fecal calcium losses are 40mg/day making the total required ~216 mg/day. With a fractional absorption at 45.6% the estimated total intake required is 474mg/day (42). This formed the basis for the calcium EAR for children 1-3 years of age, rounding to 500mg/day (7). In an assumption by Lynch and colleagues an additional 30% calcium retention would meet the needs for 97.5 % of the population (42). This was calculated as 180mg/day and is based on calcium absorptive efficiently resulting in the RDA of 700mg/day (7).

In North American adults and children, approximately 70% of dietary calcium comes from milk and other dairy products (1). The remaining calcium comes from vegetables (7%), grains (5%), legumes (4%), fruit (3%) and meat, poultry fish and eggs (5%)(7). When comparing the bioavailability of calcium in different foods, studies show that calcium naturally occurring in cows' milk is more easily absorbed than calcium found in plant sources (9, 17, 41). Plants such as spinach contain high levels of oxalate which is believed to inhibit intestinal calcium absorption (43). Furthermore, other nutrients found in cows' milk act synergistically to promote calcium absorption, these include vitamin D, lactose and casein phosphopeptides (43). Cows' milk also provides dietary phosphorus which is essential for bone development (40). Phosphorus binds with calcium to create hydroxyapatite, the substance making up 70% of bone composition (7).
Calcium contained within bone constitutes 99% of body calcium stores (1). The remaining 1% is found in the extra cellular fluid and soft tissues (44). Calcium and phosphorus form hydroxyapatite crystals in the matrix of the bones (7). Hydroxyapatite, written as \( \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 \) contains a calcium-phosphorus ratio of approximately 1.5 (1, 7, 40). Bone calcium has two roles. First, it is the main component of bone structure and second, it serves as a calcium reservoir, offering an available calcium source should blood calcium concentration decline (1). As growth continues, the crystals become denser providing strength and rigidity to the maturing skeleton (7). Calcium balance is especially important during this period (14).

Maintaining adequate levels of circulating calcium is not only important for bone growth but critical for the body to function normally (7). Calcitonin and Parathyroid Hormone (PTH) are involved in a feedback loop to maintain calcium homeostasis (Figure 2 (45))(1). PTH is an amino acid peptide that is synthesized by the cells of the parathyroid gland and calcitonin is an amino acid peptide that is secreted by the parafollicular cells of the parathyroid gland (44). Total calcium concentration in the blood is tightly regulated between 2.12 - 2.62 nmol/L. When blood calcium levels are below the desired concentration, calcium-sensing receptors of the parathyroid gland signal the secretion of PTH (1). PTH signals the kidney to produce calcitriol, the biologically active form of Vitamin D. Calcitriol elevates plasma ionized calcium levels by three different mechanisms: 1) Stimulation of intestinal calcium absorption, mainly in the duodenum and jejunum (45). During this process, phosphate absorption is also increased (7). 2) Liberation of calcium from bone through the stimulation of osteoclasts through a process called osteoclastogenesis (7, 45). Osteoclast precursors cells express a cell surface receptor called Receptor Activator of Nuclear factor-Kappa B (RANK) (45). Simultaneously, osteoblast cells express RANK Ligand on the extracellular surface of their plasma membrane. Osteoblast cells then combine with osteoclast precursor cells to form osteoclasts (45). PTH alone can also activate bone reabsorption via osteoclasts (1). 3) Retention of calcium by the kidneys occurs through stimulation of renal distal tubule reabsorption of calcium (7).

As serum calcium levels rise, the parathyroid gland stops production of PTH and the feedback loop is closed (1, 45). With a decrease in PTH secretion, renal tubular calcium reabsorption and osteoclastic bone reabsorption are decreased (44). In a state where calcium levels are above the desired concentration "C" cells of the thyroid gland secrete calcitonin, which works in opposition to PTH to decrease serum calcium. Calcitonin blocks bone calcium
reabsorption to ensure no more calcium is released into the blood. Concurrently, calcitriol through its receptor, VDR, provides feedback to suppress the production and release of PTH (7). Calcitriol is also influenced by serum phosphorus levels; high serum phosphorus levels suppress calcitriol, whereas low levels stimulate it (1). Through this homeostatic process bones gain and lose calcium on an ongoing basis (7). Both growing and mature bone undergo a process called bone remodeling where osteoclasts cells break down bone and osteoblasts cells build new bone (14). This continual process alters the size and shape of bones to allow for skeletal growth and helps to maintain blood calcium levels (7).

Figure 2: Parathyroid hormone (PTH), vitamin D, and calcium in extracellular fluid (ECF) feedback loop (45)
1.3.2 Vitamin D

Vitamin D is a fat-soluble steroid which is derived from both dietary sources and a sunlight mediated endogenous process (14). The two primary forms of dietary vitamin D are vitamin D$_2$ and D$_3$. Vitamin D$_2$ (ergocalciferol) is synthesized by plants and vitamin D$_3$ (cholecalciferol) is synthesized by animals (46). Humans can produce vitamin D$_3$ through a sunlight mediated endogenous process in the skin from 7-dehydrocholesterol (47). Both forms can be synthesized commercially and found in dietary supplements and fortified foods (7). Vitamin D$_2$ and D$_3$ differ in structure but when activated they have similar biological responses (47). It has been reported that vitamin D$_3$ may have a longer half-life and bind more tightly to the vitamin D Binding Protein (VDR) in the blood, raising blood concentrations more efficiently than vitamin D$_2$ (46). Once consumed, both vitamin D$_2$ and vitamin D$_3$ are hydroxylated in the liver to form 25-hydroxyvitamin D which is the main storage form of vitamin D in the body (48). A second hydroxylation takes place in the kidney to create 1,25-hydroxyvitamin D (calcitriol) which is the biologically active form of vitamin D (7, 47).

Since vitamin D is fat-soluble, it is digested and absorbed in the intestine similar to fat (1, 46). Dietary fat ingestion triggers the release of bile acids which stimulate emulsification of fats into monoglycerides and free fatty acids. Bile acids also support the formation of micelles (49). Micelles consist of aggregates including monoglycerides, ionized fatty acids and bile salts (1). Once fats are partially broken down, lypolitic enzymes break down remaining fats and fat-soluble vitamins. Micelles then carry fats and fat-soluble vitamins from the intestinal lumen into the bloodstream via passive absorption (49). Vitamin D is then stored in adipose or liver tissue (7, 47). Vitamin D absorption has been hypothesized to be most effective in the presence of dietary fat (50, 51). A clinical trial by Dawson-Huges et al. found a 32% increase in vitamin D absorption when consumed with dietary fat (50). Differences in milk fat content (ie. skim, 1%, 2%, and whole milk) may affect vitamin D absorption and ultimately vitamin D status (52). Fortified cows’ milk is the main dietary source of vitamin D for children in Canada (22) and a recent study from the TARGet Kids! group suggested that children who consumed whole milk had higher serum 25(OH)D concentration than children who consumed lower fat milk (5.4nmol/L higher for whole milk vs. 1% milk) (51).
Few foods naturally contain vitamin D. These include fatty fish, mushrooms and egg yolk (1). Cows’ milk has been fortified with vitamin D since the 1930's in North America to prevent severe vitamin D deficiency known as rickets (35). Rickets is a condition caused by vitamin D deficiency in which the bones do not calcify normally, causing bone weakness and deformation. In weight-bearing children, signs of rickets include bowed legs and beaded ribs (47). In 1965, Canada legislated the addition of vitamin D to all cows' milks, but the amount of vitamin D added to fluid milk and the method to which it was added was not standardized until 1993 (53). In Canada legislation now requires all cows’ milk to be fortified with 100 IU per 250mL cup, making fortified cow’s milk the main contributor of vitamin D (6, 22, 54). Another large contributor of vitamin D in Canada is fortified margarine (1).

The US Institute of Medicine (IOM) (now known as the Health and Medicine Division of the National Academies of Sciences) has standardized the Dietary Reference Intake (DRI) for vitamin D as the Estimated Average Requirement (EAR) and the Recommended Dietary Allowance (RDA). The Tolerable Upper Intake Levels (UL) was intended for safety (Figure 3 (7, 36)). The RDA has been defined as the recommended daily intake for 97.5% of the population to maintain serum 25-hydroxyvitamin D at 50 nmol/L with minimal sun exposure. The EAR has been defined as the median daily intake value that will meet the bone accretion, bone maintenance and bone loss requirements for half the healthy children or adults in a life-stage and gender group by maintaining a serum 25OHD at 40nmol/L (7). EAR’s for Vitamin D are consistent across age and gender groups as data new to the 2011 DRI review indicated that the dose-response relationships regarding median requirements are not significantly affected by age (7, 47) (Figure 3 (38)). Health Canada's RDA for Vitamin D for children 1-8 years of age is 600 (µg /day) and the EAR is 400 (µg /day) (38). One 250mL cup of vitamin D fortified cow milk contains ~100 µg of Vitamin D or ¼ of the EAR (37). For this life stage group specifically, the focus for determining the DRI’s is meeting the requirements for average bone accretion and positive calcium balance (7). Based on the available evidence, the IOM chose bone health as the basis for forming the vitamin D DRI’s. The committee evaluated 25(OH)D levels across several specific outcomes including calcium absorption, rickets, fracture risk and osteomalacia (7, 47). The congruence of the data link the following outcomes with 25(OH)D levels below 30nmol/L for children and adolescents, increased risk of rickets, impaired fractional calcium absorption, and decreased BMC (7, 47). For all age groups there appeared to be minimal casual evidence of
additional benefits to any of these indicators of bone health for serum 25(OH)D levels above 50 nmol/L, suggesting that this value is sufficient to be the RDA and meet the needs of 97.5% of the population. The committee determined possible extra skeletal manifestations of vitamin D such as cancer, cardiovascular disease, autoimmune disorders, infections, neuropsychological function and preeclampsia (1), but did not have strong enough evidence to inform dose-response relationships for the purposes of determining nutrient requirements. An underlying assumption made by the Institute of Medicine was that DRI's for vitamin D rest on the assumption that calcium intake meets the body's requirement. No amount of vitamin D can compensate for inadequate amounts of calcium in the diet (7).

Vitamin D, in its biologically active form, calcitriol, plays an essential role in bone health as a prerequisite for the absorption of calcium (45, 47). Calcitriol plays a regulatory role in calcium homeostasis, which is important to all aspects of bone health (1). Serum 25-hydroxyvitamin D concentration of 50 nmol/L has long been known to prevent rickets, bone deformities and poor bone mineralization (7). A recent longitudinal study demonstrated a 3.5 times higher odds of forearm fracture among children with mean 25-hydroxyvitamin D <50 nmol/L (15). Similarly, a recent case control study by the TARGet Kids! group found that children’s use of a vitamin D supplement was associated with a 2-fold reduction in odds of fracture, however they were unable to demonstrate an association between serum 25-hydroxyvitamin D concentration and fracture risk (55). Similarly, a 2015 meta-analysis and systematic review did not find sufficient evidence that 25-hydroxyvitamin D concentration <50nmol/L alone was a risk factor for childhood fracture (12).
### Vitamin D

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1 IU = 0.025 µg

**Figure 3 – DRI (RDA and EAR) for Vitamin D (36)**

1.3.4 Fat

Fat is a macronutrient that is important for brain development and as a stored energy source for growth and physical activity (1). Dietary fat also assists in the absorption of fat-soluble vitamins including vitamin D and A which are hypothesized to have positive effects on bone growth (49).
Cows' milk fats are derived from two sources, the animals' feed and the microbial activity within the rumen (56). Fats are present in fluid milk as globules which are lipoloyzed in the human mouth by lingual lipase and again in the stomach by gastric lipase (23). When fat enters the small intestine, it signals the release of cholecystokinin from mucosal cells which triggers the gallbladder to release stores of bile (56). With bile acids present, fats are then digested through the process of emulsification to monoglycerides and fatty acids which are passively absorbed by intestinal cells mainly in the proximal jejunum (1, 49). When bile salts attain a high enough concentration, they come together to form micelles. Larger fat molecules, which are unable to be passively diffused, are merged into micelles forming mixed micelles (56). Micelles transport fat-soluble vitamins and remaining fats into the intestinal cells via active transport. Finally fats and fat soluble vitamins exit the intestinal mucosa into the blood via the lymphatic system (49). Cows' milk contains a combination of fats and fat-soluble vitamins making it an efficient food source for the synergistic absorption of nutrients and fat necessary for bone growth (21).

Cows’ milk fat is one of the most complex natural fats containing more than 400 different fatty acids (21, 23, 56). Nearly all of these fatty acids are present in trace quantities with 15 fatty acids present at 1% or greater (56). Cow milk fat is composed of the following distribution: 65% saturated, 29% monounsaturated, 6% polyunsaturated and 3% trans-fat (1). Cows' milk contains an omega-6/omega-3 ratio of ~2.3 (23). Omega-3 and omega-6 fatty acids are polyunsaturated fats, a type of fat your body cannot synthesize (56). Cows’ milk is unique in that it contains 3 functional components that have been shown to have beneficial effects on health maintenance, growth and disease prevention, they include omega 3’s, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and omega-6, conjugated linoleic acid (CLA) (57). Cow’s milk is commercially available in a variety of milk fat percentages including; 0.1% (skim milk), 1%, 2%, and 3.25% (whole milk)(1).

Health Canada recommends that 30-40% or 25-35% of calories come from fat for children 1-3 years and 4-18 years of age respectively (36). The Canada Food Guide recommends that children 2-8 years of age consume 2 servings of reduced fat milk or dairy alternatives including cheese, fortified soy beverage, yogurt, and kefir, in efforts to reduce the risk of obesity (5). Although some observational studies have shown positive relationships between body fat and bone mineralization, studies in children have consistently shown an association between higher adiposity and fracture risk (14). Higher milk fat consumed in childhood might be associated with
higher energy intake, higher body weight and increased fracture risk. However, recent research has suggested that this may not be true. Vanderhout and colleagues from the TARGet Kids! group identified that low-fat milk consumption was associated with lower vitamin D stores and higher childhood adiposity (51). The association between vitamin D and bone health is well established and vitamin D is a fat-soluble vitamin therefore it is cows’ milk fat may play an important role in promoting bone health and preventing fractures in childhood.

1.3.5 Protein

Proteins are macronutrients comprised of 20 amino acids (AA), 9 essential (meaning the body needs to ingest them) and 11 non-essential (meaning the body can synthesize them) (1). During childhood, protein fuels the growth of cells that comprise organs, muscles, and skin. If energy intake is inadequate, dietary protein is used for energy and not available for tissue growth and synthesis of essential proteins (58). Prospective observational studies have suggested that protein intake was associated with bone mineral mass acquisition, mainly before puberty (13). Protein ingestion stimulates the osteotropic hormone-insulin-like growth factor (IGF-I), which is a precursor in the bone-building pathway and important for bone mass accumulation and longitudinal bone growth (17). IGF-I stimulates the kidneys and increases the circulating levels of calcitriol, in turn boosting the intestinal absorption of calcium and phosphate, the main components of hydroxyapatite. IGF-I at the kidney also increases tubular reabsorption of phosphate. This dual actively of IGF-I increases the concentration of calcium and phosphate and therefore positively influences the process of bone mineralization (14). Dietary protein also provides the growing body with amino acids needed for creating the bone matrix (17). Collagen (Type I) represents about 98 % of total bone proteins, the remaining bone proteins are non-collagenous (14). To build bone, cells first lay down a matrix comprised of protein collagen that is filled later with hydroxyapatite crystals (1). In well-nourished children variations in protein intake within the normal range can be associated with modifications in skeletal growth, thereby modulating the genetic potential in peak bone mass attainment (13). In a longitudinal study on healthy boys and girls aged 6-18 year of age, protein intake accounted for 3-4% of the variance in measures of bone strength after accounting for genetics (59). Furthermore one study showed that higher protein intake can enhance the positive influence of physical activity on BMD in pre-
pubertal children (13). However, BMD or BMC are surrogate measures of bone strength, whereas fracture incidence is the key functional outcome (17).

Protein digestion starts in the stomach where hydrochloric acid denatures (uncoils) the proteins, simultaneously hydrochloric acid activates the digestive enzyme pepsin (1). These partially broken-down proteins are also digested by pepsin and broken down into polypeptides and amino acids. Once the polypeptides enter the small intestine, they are further broken down by various pancreatic enzymes. Finally, peptidase located on the membrane of the intestinal surface splits the remaining short peptides into amino acids (60). Membrane carriers then transport amino acids into the intestinal cells where they are used for energy to synthesize needed compounds such as a bone matrix (1, 60). Unused amino acids travel to the liver for storage (1).

One cup (250mL) of cows' milk provides approximately 8g of protein (37) and Health Canada's "Dietary Reference Intakes" recommends 13g of protein per day for a child 1-3 years of age. This increases to 19g for children 4-8 years of age (38).

Dietary protein DRI's were based on evidence from studies considering nitrogen balance and physiological protein intake to meet the needs of 97-98% of the population. For children, results from these studies were fractioned per kilogram of body weight to define the requirements for protein intake during growth (58). High-quality proteins provide the body with all the essential amino acids to support growth. The two main factors that influence protein quality are the protein's digestibility and its amino acid composition (1). Protein from cows’ milk is regarded as high quality based on human amino acid requirements, digestibility and bioavailability (17). Cow milk is comprised of 2 main proteins: soluble whey which represents 20% of milk protein, and insoluble casein which represents 80% (60). Casein is a carrier for calcium and phosphorus which are important for bone health. Casein transports calcium and phosphorus by forming coagulum which improves their digestibility (21). Coagulum is a combination of fat, protein and other nutrients merged to together for ease of nutrient transport across barriers (49). Whey protein plays an important role in immune function as it contains immunoglobulins (21). Whey protein, especially the fraction with an alkaline isoelectric point (milk basic protein (MBP)), has been shown to suppress urine levels of bone reabsorption markers and increase BMD (9). Furthermore, aromatic amino acids which are found in cows' milk increase IGF-I and stimulate
the intestinal absorption of calcium (61). Aromatic amino acids have been shown to down regulate osteoclastic activity which promotes bone growth (62). In growing children, milk avoidance has been associated with shorter stature and lower bone mineral mass (13).

1.3.6 Other Vitamins and Minerals

1.3.6.1 Phosphorous

Phosphorus is an important mineral for bone growth along with calcium and magnesium. Phosphorus is the second most abundant mineral in the body (1). Phosphorus and calcium combine to form generally insoluble (at physiologic neutral pH) calcium-phosphate salts whose nanocrystals solidify bone in what is called hydroxyapatite (63). Approximately 85% of the body's phosphorus is contained within hydroxyapatite crystals located in the bones and teeth (1). Hydroxyapatite is responsible for the structure and integrity of bone and maintaining adequate phosphorus, and calcium levels are essential to optimal bone growth (40). Phosphorus is found abundantly in protein-rich sources such as cows' milk (37). Cows' milk contains an optimal ratio of calcium and phosphorus with a Ca/P ratio of 1.3 (14).

1 cup of cows’ milk contains ~250mg of phosphorus (37). The RDA for children age 1-3 is 460mg/day meaning 2 cups of cows’ milk would satisfy the phosphorus RDA. The RDA climbs to 1250 mg/day during puberty when peak height velocity is reached (36). Since many foods contain phosphorus, phosphorus deficiency is uncommon in North America (1).

Phosphorus along with being a primary component in hydroxyapatite is also involved in a feedback loop to maintain calcium homeostasis (Figure 2 (45)). Certain aspects of the interaction between calcitriol and phosphorus remain unclear. What is known is that a decline in phosphate stimulates the production of calcitriol, which in turn stimulates phosphate absorption in the small intestine. Calcitriol can then induce the secretion of osteocytes in bone which result in phosphate excretion in the kidney (7).

Phosphorus is also found in other body cells. Phosphorus is a component of DNA and RNA and therefore necessary for growth (1). Phosphate also assists in energy metabolism within the high energy compound adenosine triphosphate (ATP), which contains 3 phosphate groups. Lipids can contain phosphate as phospholipids (24). The attached phosphate group helps to transport lipids into the blood. Phospholipids are the principal structure of cell membranes, and
their function is to control the transport of nutrients in and out of the cell. Lastly, proteins can also contain phosphorus in the form of phosphoproteins which are found in foods such as casein in cows' milk (21, 24).

1.3.6.2 Magnesium

Magnesium is a cation within the body's cells (24). Magnesium is the 3rd leading mineral component in bone. One gram or less if found in the body, and 50% of that is present in the bones. Similar to calcium, bone magnesium may serve as a reservoir to ensure normal blood concentrations. Good sources of magnesium include legumes, seeds, nuts, fish and cows' milk (1). Magnesium is sensitive to the acid pH environment of milk processing. Therefore, Mg concentration is variable; however, cows' milk is still considered a good source of magnesium (24). 1 cup of cows’ milk contains 20 -30 mg of magnesium (37) and the RDA for children 1-3 years of age is 80mg/day (36).

A known role for magnesium aside from bone health is as a catalyst in the reaction that adds the last phosphate to the high energy compound ATP. This process is essential to the body’s use of glucose, the synthesis of proteins, fat and nucleic acids and cell membrane transport systems (24). Magnesium also works in opposition to calcium in muscle contraction and blood coagulation (1).

1.3.6.3 Vitamin A

Vitamin A is a fat-soluble vitamin and is present in the body in 3 forms, retinol, retinal and retinoic acid (24). Vitamin A is essential for growth and development specifically in immunity, eye health, and bone development (21). In developing countries where vitamin A sources are plant carotenoids, vitamin A deficiency is high (14). The main bioavailable sources of vitamin A are preformed vitamin A from animal-based foods such as cows' milk (14).

The RDA for vitamin A for children aged 1-3 is 300µg/day (36). 1 cup of cows' milk contains ~140µg. Therefore 2 cups of cows' milk will ensure children aged 1-3 meet their daily recommended dietary allowance (37). Vitamin A for bone health appears to have a U-shaped relationship with a relatively narrow optimal dosage window with low and high intakes associated with adverse bone outcomes including bone loss and increased risk of fracture in adulthood (14). Vitamin A is versatile and known to regulate the expression of several hundred
genes. Its primary roles include promoting vision, participating in protein synthesis and cell differentiation and supporting reproduction and growth (1).

1.3.6.4 Potassium

Another potentially growth limiting nutrient found in cows' milk is Potassium (21). Potassium is not abundant in many foods, but is found in green and root vegetables, fruits, legumes and cows' milk (1). Potassium is important in bone health as a component of the process that maintains calcium homeostasis, particularly urinary calcium conservation and excretion. Low potassium diets are known to increase urinary calcium loss, and diets high in potassium are known to decrease this. Finally, potassium is believed to work in opposition to sodium, minimizing the detrimental effect of sodium consumption on urinary calcium excretion (14). Other functions of potassium include blood pressure regulation, muscle contraction and ionic equilibrium (24).

2. Childhood Fracture

2.1 Bone Formation

Bone formation begins prenatally through two processes; endochondral ossification and intramembranous ossification (58). Intramembranous ossification occurs by mesenchymal cells which differentiate directly into osteoblasts. Although this process is generally associated with embryonic development it can also occur postnatally (64). Endochondral ossification occurs through a hyaline cartilage template where cartilage is slowly replaced by mineralized bone tissue as the child grows (58, 64). During childhood, the immature woven bone tissue is replaced by mature bone, referred to as "Lamellar" bone (65). This process continues through adolescence until adult bone replaces cartilage by the third decade of life. Bone mineralization begins and ends earlier in girls than boys (58). The shape and size of the skeleton are mainly determined genetically, but some variation in size is determined by environmental factors including nutrition (14).

Long bones grow in length as a function of the epiphyseal disc, commonly known as the growth plate (58). The growth plate is a narrow band of cartilage cells that separate the epiphysis (the round end of the bone) from the diaphysis (long shaft of the bone) which forms in utero as the primary ossification center (58, 65). Growth occurs in this proliferating zone by the
combined effect of cartilage cells multiplying and expansion of the intercellular matrix (65). At birth, two secondary ossification centers form at the proximal and distal ends of the long bones (58). Through childhood, bones remodel and grow until the closure of the distal and proximal growth plates occurs through a process called epiphyseal union, signaling the end of bone growth (65). Long bones are unique in that their widest part is at each end. As the bone grows longitudinally, there is simultaneous modeling activity that preserves the bones shape (65).

The rate of bone growth varies on an individual basis, however, on average there is an increase in bone growth in the first 2 years of life, a plateauing of growth between 2 years of age and puberty and a sharp increase again at puberty. Across all growth periods, BMD increases linearly (58). Nutritional intake is one of the most important environmental factors that influence bone mass accumulated during childhood, and cows' milk is rich in nutrients that are believed to be important for bone growth including calcium, protein and vitamin D (9, 17).

2.2 Bone Cells and Tissue

Two types of bone tissues are found in all bones; cortical and cancellous bone (65). Cortical bone is found in the shafts of long bones and is made of overlapping cylindrical units. Cancellous bone, commonly referred to as spongy bone, is located at the ends of long bones (64). Bones are composed of cells and extracellular matrix (ECM)(65). The ECM is subdivided into organic and inorganic parts. The organic matrix is mainly comprised of type 1 collagen, proteins and proteoglycans. The inorganic matrix is made up of calcium and phosphorus deposited into the collagenous matrix in the form of hydroxyapatite (64).

Bone tissue is hard, durable and made to withstand substantial loads and impact from physical activity (63). Bone tissues also serve as a reservoir of important minerals such as calcium and phosphate. The hardness of bone comes from its protein-rich interconnected organic matrix that provides a scaffold for mineral deposition (63). The mineralization of this extracellular matrix by calcium and phosphate form hydroxyapatite which give strength and rigidity. The bone matrix is also the body’s main store of these minerals (7). Bone accretion during childhood (2-8 years) is proportional to the rate of growth. During this period, bone mineralization and growth in length are in equilibrium (14). A deficiency in calcium or phosphate during childhood could disrupt this equilibrium and leave bones susceptible to fracture at a particularity sensitive time as childhood precedes adolescence, the period of peak fracture
incidence (66). Therefore, dietary sufficiency in childhood, of which cows’ milk has been proposed to be an important component, is important for building fracture resistant bones. A randomized control trial involving 757 pre-pubertal girls from China showed significantly higher gains in BMC and BMD among the girls served cows’ milk daily when compared to the control group who were not provided daily cows’ milk (67).

2.3 Bone Remodeling

Bone remodeling is a continuous process that builds and repairs bone (65). During childhood and adolescence, bone remodeling aids in the growth and shaping of bones (58). Bone remodeling involves 4 main processes: activation, resorption, formation and resting (14). This process is regulated by endocrine and paracrine factors including, but not limited to, parathyroid hormone, vitamin D, insulin-like growth factor and receptor activator of nuclear factor kappa B (14).

Osteoclasts and osteoblasts are the cells involved in bone remodeling (3). The homeostatic balance between each cell type is important for bone health during childhood and adolescence (1). Osteoclasts, derived from hematopoietic precursor cells in the granulocyte-macrophage lineage, are the only cells capable of breaking down mineralized bone. They require two signals for activation: an increase in the number of macrophages and that osteoclast precursors cells display RANK (68). Activated osteoclasts fuse to form mature bone resorbing cells and are recruited to the activated surface. Osteoclasts attach tightly to the surface creating an acidic environment to dissolve the inorganic matrix releasing calcium and phosphorus into the blood (14, 68). The resorption phase takes about 3-4 weeks to complete (64). Osteoclasts then die by apoptosis and disappear from the activated surfaces (68). Osteoclastic activity can be quickly stopped by Calcitonin. This process has been hypothesized as an emergency brake to protect against hypercalcemia rather than a general mechanism for stopping osteoclastic activity (7).

Multipotent mesenchymal stem cells undergo differentiation to create bone-building osteoblasts which synthesize new organic matrix (14). This formation step requires adequate amounts of calcium and phosphorus and takes about 3-4 months to complete. The new bone surface remains dormant in the resting phase until the next cycle beings (64). As osteoblasts synthesize bone they become trapped in the matrix and form mechanoreceptors called osteocytes. These cells regulate the flow of nutrients and minerals between the blood and the
bone matrix (4). In response to damage or stress, osteocytes release substances which influence the bone remodeling process (68).

Peak bone mass occurs in adolescence (58). Dietary calcium and its absorption need to be at or above a threshold level to satisfy skeletal modeling while taking into account losses through urine, feces, and sweat (14). It has been suggested that low dietary calcium intake during childhood could contribute to reduced peak bone mass and bone fragility fractures later in life (69, 70). Cows’ milk is one of the most abundant sources of dietary calcium per calorie (1, 9). Drinking the recommended 2 cups of cow’s milk per day provides children with 60% of the calcium RDA (37).

Bone remodeling is greatest during periods of growth but continues throughout the lifespan as a functional adaptation of the bones to repair from stress (14). Each year 10% of the skeleton is replaced, therefore, every 10 years the skeleton is completely renewed (65). Many observational studies and RTC's have shown positive associations between cows’ milk intake and bone turn over markers (17).

2.4 Childhood Fracture Incidence

Fractures are common among healthy children (2). Children who experience a first fracture at a young age are at higher risk of becoming adolescents and adults who fracture a bone (71). The incidence of childhood fracture is increasing (2, 72, 73). Over the last 30 years, there has been an increase in low-energy trauma fractures, specifically forearm fractures during childhood (72). Distal forearm fractures account for 25-30% of all pediatric fractures (2, 16). Approximately 1/3rd of girls and ½ of boys will fracture a bone by their 18th birthday (74, 75). However, only 10% of children will fracture before 10 years of age (72). In Ontario, Canada a recent study identified that 24% fractured a bone before 15 years of age and 4% had 2 fractures (76). Fracture incidence increases in both sexes during puberty while Peak Height Velocity (PHV) is greatest (72). During puberty, bone length surpasses the speed at which bones can mineralize, resulting in a period of relative skeletal weakness (77). This weakness in part is caused by a draw on minerals within the cortical bone to meet the mineral demands of the growing skeleton (66). Simultaneously, physical activity increases creating a scenario where bones are more likely to fracture (16).
2.4.1 Pre-Pubertal Fracture

Bone quality changes as children progress into puberty and bones are more stable in the pre-pubertal stage (15). A retrospective cohort study involving 90 children from New Zealand, which focused on multiple fractures found that participants who first fractured a bone before the age of 10 years had a higher likelihood of multiple fractures than children who first fractured after the age of 10 years (78). Children's bones are not as rigid and not exposed to the same forces as adolescent bones resulting in fewer fractures (58). As fractures are less common in pre-pubertal children, bone fracture may signify skeletal weakness and be more indicative of nutritional deficiency than adolescent fracture (2, 12). Ryan and colleges found a significantly higher proportion of fractures due to minor trauma in the 10-14 age group when compared to the 5-9 age group (16). These results suggested that childhood bones are more fracture resistant, especially due to minor trauma (16, 77). Therefore, when pre-pubertal children do fracture there may be a preventable underlying cause such as nutritional deficiency. A recent systematic review and meta-analysis of observational studies suggested that pre-pubertal fractures may be more sensitive to nutritional factors such as increased sugar-sweetened beverage consumption and cows’ milk avoidance, more so than adolescent fractures (12, 16, 71).

2.5 Risk Factors

Emerging research is supporting that obesity, physical activity, dietary factors, and vitamin D are independent predictors of childhood fracture (79). Although a recent systematic review and meta-analysis concluded that fracture risk seemed to be associated with milk avoidance, children who do not consume cow’s milk may do so for a variety of reasons including health issues and allergy which may themselves be risk factors for fracture (12).

2.5.1 Non-Nutritional Risk Factors

Age and Gender

Fractures are most common in adolescence and in the elderly (80). The peak age of childhood fracture is 14 years for boys and 11 for girls (79). Fracture incidence, and therefore fracture risk, increases with age until the end of puberty. Boys consistently have a higher fracture
risk when compared to girls of all ages (79). Approximately 30% of girls and 50% of boys fracture a bone before the end of childhood (74, 75).

**Ethnicity**

The relationship between childhood fracture and ethnicity is debated in the literature and is an emerging area of research (81). A recent prospective cohort study demonstrated that girls and boys of European descent had double the risk of fracture when compared with children of other ethnic backgrounds (82). To address the underlying mechanism for this ethnic difference, one study suggested that African American and Hispanic children had significantly higher bone strength when compared to Caucasian children (83). Based on peripheral quantitative computed tomography, African American and Hispanic children had greater bone volumetric density, suggesting that these differences in bone density could result in a reduced risk of fracture (83).

**Socio-Economic Status**

Socio-economic status and its relationship with fracture risk has also been debated in the literature (79). One study from Glasgow found that children from families with higher socioeconomic status had higher rates of fracture, suggesting access to organized sports increased their risk (84). Another from Wales found no difference in fracture risk across all levels of socioeconomic status (85). The authors suggested the observed effects depended on individual-level factors such as the fracture mechanism rather than socio-economic status per se (85).

**Adiposity**

Higher child adiposity is believed to be associated with higher risk of childhood fracture. While weight-bearing exercise is good for bone mineralization, increased weight status may affect balance increasing the risk of falls causing bones to fracture (16). Fracture risk among heavier children may also be higher as they fall with greater force from any given elevation (16). One study has shown an increase in morbidity from childhood fracture among children with obesity (86). A recent systemic review which included over 1 million children from ages 0 to 19 from 19 countries found that higher adiposity was associated with an increased risk of lower extremity fractures (87). Associations among obesity and upper extremity fractures are inconsistent (79). A study from New Zealand on 1037 children reported an association between
increased body weight and childhood fracture among pre-pubertal children (88). It has been hypothesized that a U-shaped relationship between adiposity and childhood fracture exists (79). Children at high and low ends of the adiposity spectrum may be at greatest risk of fracture (89). A 2013 mechanistic study which focused on pelvic fractures in 10 year old children identified that the force which children with obesity fall took precedence over the cushioning effect of excess subcutaneous adipose tissue (90). On the other end of the adiposity spectrum, children with very low adiposity may have lower bone mineralization and increased skeletal fragility (90). Similarly, a 2014 case control study involving 418 girls identified that those with anorexia nervosa or who were severely underweight were at an increased risk of fracture, especially from minor trauma, when compared to the normal weight controls (91).

*Bone Mass/BMD/BMC*

The relationships between bone mass and fracture risk is well established in the literature. Bone mass, bone mineral density (BMD) and bone mineral content (BMC) are measurements of the amount of bone mineral within bone and are commonly used as a proxy measures for fracture (18). BMD/BMC are important because they are proportional to fracture resistance in adults and children (14). There is evidence from prospective cohort studies that low bone mass and lower BMD are risk factors for childhood fracture (79). The largest prospective study, consisting of 6213 children aged ~10 years old followed for 24 months, showed that per standard deviation (SD) lower bone mass, fracture risk was greater by 89% (92). Another case control study that examined bone mass and size of children with fracture and recurrent fracture found that lower bone mass than expected for body size contributed to an increased risk of fracture (93). A 1998 case control study which involved 200 Caucasian girls aged 3-15 from New Zealand concluded that low bone density was more common among girls with forearm fractures than in those who have never broken a bone, suggesting that low bone density may contribute to fracture risk in childhood (94). Another study which involved 97 adolescents from Canada focused on the peak incidence of fractures during puberty and found that BMD decreases significantly during the period of PHV, supporting the theory that lower BMD causes skeletal weakness which increases fracture risk (66).

The volume of cows' milk consumed has been associated with measures of bone turn over, BMC, and BMD in observational studies (17, 18). A 2008 meta-analysis of 21 RTC’s on
dairy/calcium consumption on BMD in children found that higher dairy/calcium intake with or without vitamin D increased both BMC and BMD in children with low baseline intakes (18). However, it is unclear whether the relationship between cows’ milk consumption and BMD/BMC translates beyond these measures of bone strength to decreased fracture risk (12).

*Physical Activity*

Sports and play contribute to the most fracture events with play dominating in the first decade of life (80). There are likely two opposing mechanism behind the relationship between physical activity and fracture risk (79). First is the benefit of weight-bearing activity in stimulating osteogenic activity and bone turnover. Second is the exposure to high impact activities that could increase the risk of fracture (79). The increase in high impact physical activity as the child ages correlates well with the increase in fracture risk with age (80). Overall, there is a U-shaped relationship with children at the lowest and highest ends of physical activity at greatest risk of fracture (72). Low levels of physical activity results in low bone mass, size and strength, increasing risk of fracture while high levels of physical activity outweigh the beneficial effects of osteogenic stimuli on bone strength (79). One Finish study has suggested the decline in physical activity overall results in higher prevalence of overweight an obesity and this has an opposing effect on the relationship between physical activity and fracture risk (72). A Randomized Control Trial (RTC) conduced in Sweden which tested the effect of a moderate intensity 40 minute per day exercise program over 5 years on 1587 pre and post-pubertal children showed that the exercise intervention group had higher bone mass and size without an increase in fracture risk (95).

*2.5.2 Nutritional Risk Factors*

*Cows’ Milk Avoidance*

It has been well established that milk avoidance is a risk factor for childhood fracture (12). Children avoid cows’ milk for various reasons including; cows’ milk protein allergy (CMPA), lactose intolerance, cultural beliefs and taste preferences (96). Children who avoid cows’ milk may have dietary calcium intakes below the recommended dietary intake (RDI) and have an increased risk of fracture (59). A recent case control study showed a 4.6 OR of fracture among children with cows’ milk allergy (20). The main reason for cows’ milk avoidance is adverse
reaction to milk ingestion which can be due to lactose intolerance or, more rarely, cows' milk protein allergy (60). Lactose is the main carbohydrate in milk, and it is a disaccharide comprised of glucose and galactose (22). Lactose intolerance is present when the enzyme Beta-galactose is deficient. If cows' milk is ingested into a host who is deficient in Beta-galactose, there will be several GI symptoms including bloating, nausea and diarrhea (60). CMPA is a food allergy observed in 2 - 7% of children and it is either developed in utero or during the first year of life (20). It tends to remit during childhood and is uncommon in adults (60). CMPA is associated with elevated immunoglobulin E (IgE) serum concentration, and the symptoms can be immediate (IgE-mediated) or delayed (non-IgE-mediated) (22). As it primarily affects young children, growth can be compromised (60).

Many observational studies have identified an increased risk of fracture among children who do not consume cows' milk (12, 19, 97). Similarly, children with more than one fracture are overrepresented in the population of children who avoid milk for any reason (78, 98). The underlying mechanisms for the increased risk of fracture among children who avoid milk has been under considerable debate making such studies challenging to interpret (20). On one hand, children who avoid cows' milk may have lower calcium and vitamin D intake which results in lower BMD and lower peak bone mass resulting in weaker bones (99). On the other hand, the underlying reason for milk avoidance itself may be associated with weaker bones (100). The authors of a recent paper discussed that a possible underlying mechanism is that CMPA and lactose intolerance, which are the two most common reasons for milk avoidance, cause chronic diarrhea and therefore malabsorption of calcium and vitamin D (101). Lower calcium and vitamin D absorption may impair bone mineralization increasing the risk of childhood fracture. What these studies have not addressed is whether volume of milk consumed by healthy children is related to fracture risk.

**Calcium**

The relationship between calcium intake and bone health is a well-established concept (7). A 2008 systematic review of RTC's involving 3821 children (83% female) between the ages of 4 - 17 years showed that higher calcium/dairy product consumption resulted in higher total body BMD and lumbar spine BMD in children with low baseline intakes (18). However, studies which have directly evaluated the relationship between calcium intake and childhood fracture show
conflicting results (12, 79). A 2015 systematic review and meta-analysis of observational studies found 3 case-control studies judged to be of high quality which had contradictory results (12). The first showed that among 76 African American children age 5-9 years those with bone fracture had higher calcium intakes relative to controls without bone fracture (15). The second which involved 1412 children age 4-16 years from Finland found that lower dietary calcium intake was more common among fracture cases than non-fracture controls (102). The third study which involved 200 girls age 3-7 from New Zealand did not identify a difference in calcium intake between fracture cases and age matched controls (94). One author suggested that the association between increased risk of fracture and calcium intake maybe be due to higher total caloric intake, and therefore higher calcium intake, among children with fracture (15). Authors who have supported increasing calcium intake for fracture prevention have suggested that the bioavailability of calcium from dairy products is more beneficial for bone outcomes than calcium supplementation alone or calcium from other sources (17). Further, the simultaneous intake of phosphorus and vitamin D which are found in cows’ milk are believed to aid in the absorption and usage of calcium (9).

Sugar Sweetened Beverage Consumption

A positive relationship between sugar-sweetened beverage consumption and higher childhood fracture risk has been consistently reported. A recent systematic review and meta-analysis of observational studies on early nutrition and childhood fracture identified that higher sugar-sweetened beverage consumption was associated with higher fracture risk among children 9-13 years of age (12). A case control study by Petridou and colleagues involving 100 children from Greece identified that higher intake of sugar-sweetened beverages such as non-cola fruit drinks and cola beverages was associated with higher risk of fracture (103). Ma and colleagues also identified that cola, but not total carbonated beverage consumption was associated with increased wrist and forearm fracture in among 128 children age 9-16 years from Australia. This association appeared to be mediated by higher screen time and lower BMD but not by lower cows' milk consumption (104). Similarly, Petridou found no effect of cows' milk consumption on the relationship between cola, fruit juices and childhood fracture risk (103).

Vitamin D
Similar to calcium, the physiology of vitamin D and bone health is well established and is the basis for the DRI for vitamin D and calcium in Canada (7). Paradoxically, the relationship between vitamin D intake and childhood fracture risk is unclear. One case-control study involving 150 pre-pubertal children aged 5-9 years from the United States found that cases with fracture were more likely to have lower vitamin D serum levels than controls (15). Another case-control study conducted by the TARGet Kids! group found that vitamin D supplementation, but not serum 25 hydroxyvitamin D levels or vitamin D fortified cows' milk intake, was associated with a reduction in fracture risk (55). Similarly, Mäyränpää et al. conducted a case-control study involving 1412 children and found no difference in mean serum 25 hydroxyvitamin D levels between fracture cases and controls when measured at a mean age of 10.7 years (102). Lastly, in relation to cows' milk, a 2008 meta-analysis of RTC’s which focused on dairy intake and BMC as a proxy measure for fracture, found that higher dairy and calcium intake increased BMC regardless of whether the dietary source was fortified with vitamin D (18).

2.6 Fracture Risk and Milk Volume

Cows' milk consumption has been widely publicized as being good for our bones (9, 17). However, the relationship between the volume of cows’ milk consumed in childhood and fracture risk is remarkably unclear (8). One case-control study which involved 150 children age 4-16 years from England with recurrent fracture identified that cows' milk intake (liters/day) was lower among children with recurrent fracture as opposed to single fracture and the control group (93). The authors speculate that the mechanism to which low cows’ milk may increase the risk of recurrent fracture is that low milk intake may increase bone remodeling and decrease bone formation via a lack of anabolic growth factors such as IGF-1 (93). Similarly, in a prospective study involving 3251 adults (20-49 years of age) from the United States who consumed milk less than once per week as children (5-12 years of age) were at higher risk for osteoporosis and osteoporotic fractures than adults who consumed milk daily as children ($P < 0.05$) (105).

A 2015 systematic review and meta-analysis of observational studies focused on early nutrient intake and fracture risk in childhood identified 2 case-control studies judged to be of high quality that evaluated the volume of cows' milk consumed and fracture risk in healthy children aged 2-13 years (12, 15, 104). The first was a 2004 study conducted in Australia on 412 children age 9-16 years. The authors found no difference between cows' milk consumption in
fracture cases and controls (104). The second study included 76 fracture cases and 74 controls
age 5-9 years from the United States and also found no difference in mean weekly number of
milk servings between cases and controls (12). The authors of the systematic review and meta-
analysis concluded that insufficient evidence was available to determine if cows' milk
consumption in childhood is protective against childhood fractures (12). Another study by Lanou
and colleagues (non-systematically) reviewed the literature on the relationship between dietary
calcium, dairy product consumption and bone health in children (8). They also did not identify
studies which demonstrated a positive association between cows’ milk intake and fracture
prevention in childhood. They concluded that current guidelines focused on increasing milk
consumption for fracture prevention or promoting bone health have little merit (8). To my
knowledge, no prospective studies have examined the relationship between the volume of cows' milk consumed in healthy children and fracture risk.

2.7 Fracture Risk Milk Fat Content

A relationship between cows' milk fat consumption and fracture risk has been hypothesized
but little studied (52). The proposed biological mechanism is that calcium and vitamin D
absorption, which have been shown support bone health and potentially reduce fracture risk (12,
18, 79), may be enhanced if consumed with dietary fat (50, 51, 106). One recent study from the
TARGet Kids! group showed that children who consumed whole cows’ milk (3.25% fat) had
higher serum 25 hydroxyvitamin D levels and lower adiposity when compared to children
drinking 1% cow's milk which both may be associated with lower fracture risk (51). To my
knowledge, no prospective cohort studies have examined the relationship between the fat content
of cows' milk consumed and fracture risk in children.

3. Summary of Literature Review

Childhood fracture has been increasing over the last 30 years, specifically childhood forearm
fracture from low impact mechanisms, suggesting there may be an underlying preventable reason
for fracture (2, 73). Dietary habits are known to have a considerable influence on proper bone
growth, development, and potentially fracture (100). Cows’ milk plays an important role in
childhood growth including associations with height which is commonly used as a marker for
overall health (107). Cows’ milk is the main dietary source of calcium, vitamin D and other nutrients proposed for optimal bone growth (9, 108). However, studies on the association between cows’ milk consumption and fracture risk are few with inconsistent findings. None the less, many organizations promote cows’ milk as a component of a healthy diet and current guidelines state that children 1-3 years of age should be consuming 2 cups of lower fat milk daily for optimal healthy growth (109). Further, approximately 88 % of Canadian children aged 1-3 consume cows’ milk daily (11). Milk avoidance has been previously associated with an increased risk of fracture and lower BMD (12, 110), however underlying causes such as diarrhea caused by CMPA may be causing the increase in fracture risk (101). As well, low milk consumption has been associated with decreased BMC and recurrent fracture, but not increased the risk of first fracture (18, 93). However, no studies have prospectively evaluated the association between cows’ milk volume or cows’ milk fat consumed and childhood fracture risk.

Studies on the relationship between cows’ milk intake and childhood fracture risk are limited and have reported inconstant findings. (8, 17, 20). Further, there has been no research on the relationship between milk fat percentage and fracture risk. There is a growing body of literature is raising doubts about the role cow’s milk plays in fracture prevention (8, 20, 52). Further research is needed to evaluate whether cows’ milk consumption and milk fat content during childhood provides fracture prevention benefit. The objectives of this study are: to evaluate whether volume of cows' milk consumed between 1 and 3 years of age is associated with risk of fracture between 3 and 10 years of age, to explore whether milk-fat consumed between 1 and 3 years of age is associated with risk of fracture between 3 and 10 years of age and explore whether the milk-fat content consumed modifies the relationship between milk volume and fracture risk.
Chapter 3: Milk volume, milk fat and childhood fracture
Early childhood cow’s milk consumption and fracture risk: A prospective cohort study

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Abbreviations: TARGet Kids!, The Applied Research Group for Kids; zBMI, body mass index (BMI) z-score.

Table of Contents Summary: This prospective cohort study which examined childhood cow’s milk consumption and fracture risk, found no association to support increasing milk consumption for fracture prevention.

What is Known on This Subject: Childhood fractures precede future fractures and osteoporosis in adulthood. Childhood cows’ milk consumption has been associated with increased markers of bone strength, however no studies have prospectively examined the association between early childhood cows’ milk consumption and fracture risk.
**What this Study Ads:** Higher cow’s milk consumption in early childhood was not associated with a reduced risk of fractures in later childhood. Findings from this study do not support efforts to increase childhood cow’s milk consumption for the purpose of fracture prevention.
Contributors Statement Page

Ms. Allison and Dr. Maguire designed the study, performed the statistical analyses, composed the draft of the manuscript, and were responsible for the final content of the manuscript.

Dr. Lebovic assisted in the research design, reviewed and revised the manuscript and assisted in the statistical design and analysis.

Dr. L’Abbe, Dr. Howard, Ms. Morency and Dr. Birkin assisted in the research design and reviewed and revised the manuscript.

All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.
1. Abstract

**Background and Objectives:** Cow’s milk is consumed by most North American children yet the relationships between the volume and fat content of cow’s milk consumed and childhood fracture risk are unclear. The primary objective of this study was to evaluate whether volume of cow’s milk consumed between 1-3 years of age was associated with risk of fracture between 3-10 years of age. Secondary objectives explored whether milk-fat was associated with risk of fracture and whether milk-fat content modified the relationship between milk volume and fracture.

**Design:** This was a prospective cohort study of 2466 children. Children who had conditions affecting growth, chronic illness, or severe development delay were excluded. The primary exposure was volume of cow’s milk consumed between 1-3 years and the secondary exposure was the average percentage of milk-fat consumed by each child over the same period. The primary outcome was parent report of fracture experienced between 3-10 years.

**Results:** In the primary and secondary adjusted analysis, an association between milk volume and fracture risk and milk fat content and fracture risk was not observed, (aRR= 1.04; 95%CI:0.87-1.26 per cup of cows’ milk consumed) (aRR= 1.05; 95%CI:0.84-1.31 per 1% milk fat). Cow’s milk-fat did not modify the relationship between milk volume and fracture. (p= 0.24).

**Conclusions:** In this study we did not identify a protective effect of early childhood cow’s milk volume or fat consumption on fracture risk in later childhood. Future prospective research is needed to understand whether cow’s milk is beneficial for fracture prevention through the life course.

**Keywords:** Cow’s Keywords: Cow’s milk, nutrition, fracture risk, childhood, pre-pubertal, prospective studies
2. Introduction

One-third of children in North America fracture a bone by the end of childhood (74, 75). During the last two decades, the frequency of childhood fractures have increased by one third, causing significant hardship and pain (2, 16). Childhood fracture has been associated with an increased risk of adolescent and adulthood fractures as well as osteoporosis later in life (2, 71, 74, 79).

Several risk factors for childhood fracture have been identified including older age, male sex, high and low levels of physical activity, greater adiposity and nutritional factors such as sugar sweetened beverage consumption and milk avoidance (12, 55, 71, 75, 78, 79, 82, 93, 111). Observational studies have identified an increased risk of fractures among children who do not consume cows' milk (12, 19, 97). However, children who do not consume cows' milk may do so for various reasons including food allergy, personal preference or underlying illness which may themselves cause increased fracture risk (98, 112, 113).

Studies which have evaluated the association between the volume of cows' milk consumed and fracture risk in childhood have reported inconsistent findings (8, 12, 17). A 2015 systematic review and meta-analysis of observational studies aimed to identify dietary patterns early in life which may contribute to fracture risk among healthy children. The authors found that cows' milk consumption was not protective of fractures and identified the need for prospective cohort studies to evaluate the temporal relationship between early childhood nutritional factors such as cows’ milk consumption and childhood fracture (12).

The fat content of cows' milk may also influence fracture risk. Recent research has identified that low-fat milk consumption was associated with lower vitamin D stores and higher
childhood obesity risk (51), both of which may increase risk of childhood fracture (55, 79, 86, 87, 114).

We hypothesized that higher volume of cows' milk and higher milk-fat content consumed in early childhood would be protective against fracture in later childhood. To address this, we designed a prospective cohort study of milk consumption in early childhood and fracture risk in later childhood. The primary objective of this study was to evaluate whether volume of cows' milk consumed between 1 and 3 years of age was associated with risk of fracture between 3 and 10 years of age. Secondary objectives included exploring whether milk-fat consumed between 1 and 3 years of age was associated with risk of fracture between 3 and 10 years of age and whether milk-fat content modified the relationship between milk volume and fracture risk.
3. Methods

3.1 Study Design

A prospective cohort study was conducted through the TARGGet Kids! practice-based research network. TARGGet Kids! is a collaboration between the University of Toronto’s Faculty of Medicine and clinicians in the University’s Department of Paediatrics and Family and Community Medicine (115).

For this study, children between 1 and 3 years of age were recruited by trained research assistants at a primary healthcare visit between September 2008 and December 2016 at 9 primary healthcare clinics in Toronto, Canada and followed prospectively. Children who had conditions affecting growth (e.g. failure to thrive, cystic fibrosis), chronic illness (excluding asthma) or severe development delay were excluded.

3.2 Exposures and outcomes

Cows' milk volume and fat content were measured at the first TARGGet Kids! visit between 1 and 3 years of age which was defined as the exposure window. This window was chosen to be consistent with a 2015 systematic review and meta-analysis (12) and with recommendations from the American Academy of Pediatrics and the National Institutes of Health which recommend whole cows' milk starting at 1 year of age (4, 116). Bone fracture was measured at the last TARGGet Kids! visit between 3 and 10 years of age which defined the outcome window. This window was chosen because childhood fracture may be more sensitive to nutritional factors than adolescent fracture (12) and is known to be predictive of later fracture (16, 78). Informed consent was obtained from parents of participating children and ethical approval was obtained from the Research Ethics Board of The Hospital for Sick Children and St. Michael’s Hospital.
Trained research assistants collected data from parents at each participating practice using a standardized data collection instrument adapted from the Canadian Community Health Survey as well as anthropometric measures using standardized techniques (11).

The primary exposure variable was volume of cows' milk consumed by each child during the exposure window. Milk volume was determined by the following question “How many 250mL cups of milk does your child currently have in a typical day?” Children who did not consume cows' milk were included in the analysis as having consumed 0 cups per day.

The secondary exposure variable was milk-fat consumed by each child during the exposure window. This variable was measured by the question “Please specify your child’s diet for the past 3 days: skim, 1%, 2% or whole milk.” For subjects who consumed more than one type of milk-fat, the mean value was calculated by averaging the fat content of each type consumed (51).

The primary outcome was one or more fractures experienced during the outcome window. It was measured by the following question “Has your child ever broken a bone?” which was categorized as yes or no (92).

3.3 Other Variables

Covariates which might confound the relationship between cow milk consumption and fracture risk in childhood were determined a priori by a literature review. These variables included maternal ethnicity, household income, age, sex, body mass index (BMI) z-score, daily free play, volume of sugar sweetened beverage consumed, child multivitamin supplementation and child vitamin D supplementation.

Children's weight was measured with a precision digital scale (+/-0.025%; SECA) and height was obtained using a stadiometer (SECA). BMI was calculated as weight/height² (117).
BMI z-score was derived using the World Health Organization (WHO) growth standards, which are age and sex standardized and believed to represent children’s optimal growth (118, 119). Outdoor free play time was determined by the response to the following question “On a typical day how much time does your child spend outside or in a gymnasium for ‘recess’ or ‘unstructured’ free play?” Sugar sweetened beverage consumption was quantified by the question “How many 250mL cups of sugar sweetened beverage does your child currently have in a typical day?” Multivitamin and vitamin D supplementation were determined by the following question “Does your child take any vitamins or supplements regularly?” (Vitamin D, Multivitamin etc.). Household family income was self-reported. Maternal ethnicity was geographically categorized (120) to be consistent with Wren et al. who prospectively examined the influence of ethnicity on childhood fracture (82).

Demographic and nutritional factors including sugar sweetened beverage consumption, multivitamin and vitamin D supplementation were measured during the exposure window. Other covariates which may be associated with fracture risk including age, zBMI, outdoor free play time, and household family income were measured during the outcome window.

3.4 Statistical analysis

Population characteristics were summarized using descriptive statistics for the exposure variables, outcome variable and covariates. A modified Poisson regression model was used to evaluate the relationship between milk consumption during the exposure window and parent report of fracture during the outcome window. A sandwich estimator was used, as recommended by Zou et al., to avoid overestimated relative risk estimates which can occur when Poisson regression is applied to binomial data (121, 122). Results were exponentiated to achieve relative risk and confidence intervals.
To minimize biased $R^2$ values which can occur through data generated model building, (123) the adjusted model included all covariates listed above regardless of statistical significance. The same methods were used for the secondary analysis to evaluate the relationship between milk fat consumption during the exposure window and parent report of fracture during the outcome window (121).

To explore possible effect modification by milk-fat consumption, an interaction term between milk volume and milk fat was added to the primary model. The interaction was tested at $\alpha=0.05$ significance level using a likelihood ratio test.

Multi-collinearity was evaluated using the variance inflation factor (VIF) which was less than 5 for all covariates (124). Non-linearly was tested for in both the primary and secondary models by use of restricted cubic splines (124). Missing data appeared to be missing at random and was handled using multiple imputation (125). All variables had < 10% missing data (3.1%, 0.6% and 0.4% missing data for the primary exposure, secondary exposure and outcome respectively). All analyses were run on 50 imputed data sets and then pooled for interpretation. R version 3.31 and RStudio version 0.99.903 were used (rFoundation) (126).
4. Results

Exposure data were available on 4461 TARGet Kids! participants between 1 and 3 years of age, and outcome data were available on 2466 of these children between 3 and 10 years who were included in the analysis (1738 children were not older than 3 years of age at the time of analysis. See Figure 1). The mean age of participants during the exposure window was 1.6 years (SD 0.6) and 1137 (46%) were female. Participants consumed a mean of 1.9 cups of cows' milk per day (SD 1.1) and a mean milk fat content of 2.4% (SD 0.9) in the exposure window. Mean age of the participants during the outcome window was 5.4 years (SD 1.8) with a mean duration of follow up of 3.8 years. By the end of follow up, 153 (6.2%) children experienced at least one bone fracture (Table 1).

For the primary analysis, in both unadjusted and adjusted models, there was no statistically significant association between the volume of cows' milk consumed between 1 and 3 years of age and the risk of one or more parent reported fractures between 3 and 10 years of age (uRR=1.05; 95% CI: 0.91 to 1.18, aRR= 1.04; 95% CI: 0.87 to 1.26, per daily cup of cows’ milk consumed) (Table 2). For the secondary analysis, both unadjusted and adjusted models also did not identify a statistically significant association between cows' milk-fat consumed between 1 and 3 years of age and fracture risk between 3 and 10 years of age (uRR=0.98; 95% CI: 0.79 to 1.17, aRR= 1.05; 95% CI: 0.84 to 1.31, per 1% milk fat) (Table 2). There was no evidence of non-linear relationships between cows' milk volume or cows' milk-fat consumed and risk of one or more fractures (Figure 2 & 3). Cows' milk-fat consumption did not modify the relationship between milk volume and risk of fracture (p= 0.24).
5. Discussion

We have used prospectively collected data from a large cohort of healthy urban children to evaluate the relationship between cows' milk volume or fat content consumed in early childhood and fracture risk in later childhood. Neither milk volume nor fat content consumed in early childhood were associated with lower fracture risk in later childhood. Given that cows' milk is consumed by over 80% of North American children on a daily basis (127), and that childhood fracture is a risk factor for subsequent fractures as well as osteoporosis later in life (2, 71, 74, 79) the relationship between cows’ milk consumption and fracture risk is both of clinical and public health importance. To our knowledge no other prospective cohort studies have examined the relationship between volume of cows' milk or fat content consumed and fracture risk in children.

It has been argued that nutritional factors may play a larger role in childhood fracture than adolescent or adulthood fracture (12). Children’s bones are less dense, more porous, not as ridged and not exposed to the same forces as adolescent or adult bones (77). Whereas in adolescence, peak height velocity is reached and bone length supersedes bone mineralization while strenuous physical activity increases creating a scenario where bones are more susceptible to fracture (16, 77). Therefore, during the pre-adolescent period when bones are more stable, fractures may result from preventable underlying causes such as nutritional factors. (16).

A 2008 systematic review of randomized control trials which focused on dietary calcium intake and bone mineralization among children 4-17 years of age, found that increasing dietary calcium from dairy products resulted in increased total body and lumbar spine bone mineral density at 12-48 months follow up (18). However bone mineralization is a proxy measure for fracture and the authors concluded that further research is needed to determine whether dairy
consumption in childhood translates beyond bone mineralization to decreased childhood fracture risk (18). In a 2015 systematic review and meta-analysis of observational studies which aimed to evaluate nutritional factors that were associated with childhood fractures, 18 observational studies were included of which 2 case control studies evaluated the volume of cows' milk consumed and fracture risk in healthy children aged 2-13 years (12, 15, 104). The authors concluded that insufficient evidence was available to evaluate the effect of cows' milk consumption on childhood fracture risk (12). Lanou and colleagues reviewed the literature on the effects dietary calcium, dairy products, and bone health in children (8). The authors reported a positive relationship between dairy products and measures of bone health in children in 3 of 11 studies. One of these studies was cross sectional and 2 were randomized control trials of which neither measured the functional outcome of bone fracture. The authors concluded that “scant evidence supports nutrition guidelines focused specifically on increasing milk or other dairy product intake for promoting child and adolescent bone mineralization” (8).

A relationship between cows' milk fat consumption and fracture risk has been hypothesized but little studied (52). It has been argued that calcium and vitamin D absorption, which are believed to support bone health and reduce fracture risk (12, 18, 79), may be enhanced if consumed with dietary fat (50, 51, 106). Further, consumption of cows' milk fat has been associated with lower adiposity which is believed to be protective of fractures (51, 79, 87, 114). However, in the present study, we did not identify a relationship between milk fat consumption and childhood fracture risk.

This study had a number of strengths including the prospective design involving a large culturally diverse cohort of healthy urban children. Since cows' milk consumption was measured in early childhood and bone fractures were measured up to 10 years of age, directionality in the
hypothesized relationships was accounted for. Further, detailed questionnaire and anthropometric data allowed for adjustment of numerous potential risk factors which may have confounded a relationship between milk consumption and fracture risk.

This study had several limitations including parent-reported measures which may be susceptible to recall, social desirability or misclassification bias. While numerous potential confounders were included in the statistical models, residual confounding is a possibility. Although cows' milk is the main dietary source of calcium and vitamin D for most North American Children, we were unable account for other dietary sources of calcium due to data limitations (127). We were also unable to determine the mechanism of fracture as some may be more sensitive to nutritional factors (i.e. low impact mechanisms). Although our sample was relatively large, many children in our cohort were not yet 3 years of age at the time of analysis limiting our ability to detect a small effect. Sample size calculations using a dichotomized exposure (less than 2 cups vs. greater than or equal to 2 cups of cows’ milk consumed daily) revealed a minimum detectible risk ratio of 1.28, with 80% power given our sample size. Lastly, although the TARGet Kids! population is ethnically diverse it may not be representative of all urban children.

In the present study, neither cows’ milk volume nor fat content consumed in early childhood were associated with fracture in later childhood. These findings support a growing body of literature which suggests that efforts aimed at increasing cows' milk consumption in childhood may not result in childhood fracture prevention (8, 52, 128). Future research in young children is needed to understand whether cows' milk consumption in childhood offers benefits for fracture prevention through the life course.
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Children in TARGet Kids! 12-36 months of age
(N = 4461)a

257 (6 %) children were >120 months of age (10 years) at outcome

1738 (39 %) of children were <36 months of age (3 years) at outcome

Children with Exposure Measurement (12-36 months) and Outcome Measurement (36-120 months)
(n = 2466)b

a76 (3.1 %) of children were missing milk volume data at exposure and were imputed for analysis
b10 (0.4 %) of children were missing fracture data at outcome and were imputed for analysis
**Figure 2 - The relationship between cows' milk intake and fracture risk**

*Adjusted for child sex, age in months, household family income, maternal ethnicity, child zBMI score, hours of outdoor freeplay, multivitamin supplementation, vitamin D supplementation and sugar sweetened beverage consumption.*

**Figure 3 - The relationship between cows' milk fat content and fracture risk**

*Adjusted for child sex, age in months, household family income, maternal ethnicity, child zBMI score, hours of outdoor freeplay, multivitamin supplementation, vitamin D supplementation and sugar sweetened beverage consumption.*
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<th>Table 1: Characteristics of study participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age (months)</td>
</tr>
<tr>
<td>Sex - Female No. (%)</td>
</tr>
<tr>
<td>Cows’ Milk Consumption (Cups/Day)</td>
</tr>
<tr>
<td>Cows’ Milk-Fat Average Percentage</td>
</tr>
<tr>
<td>Consumption of sugar Sweetened Beverage (Cups/Day)</td>
</tr>
<tr>
<td>Self-reported family income</td>
</tr>
<tr>
<td>Less than $30,000</td>
</tr>
<tr>
<td>$30,000-$79,999</td>
</tr>
<tr>
<td>$80,000-$149,999</td>
</tr>
<tr>
<td>$150,000+</td>
</tr>
<tr>
<td>Child multivitamin supplementation (Yes)</td>
</tr>
<tr>
<td>Child vitamin D supplementation (Yes)</td>
</tr>
<tr>
<td>Maternal Ethnicity</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Bone Fracture</td>
</tr>
</tbody>
</table>

* Bone Fracture measured at time of outcome

49
Table 2: Adjusted Poisson regression model for volume of cows' milk and milk fat concentration at baseline and fracture at follow-up

<table>
<thead>
<tr>
<th></th>
<th>uRR** (95% CI)</th>
<th>p</th>
<th>aRR** (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows' Milk Volume (Cups/Day)</td>
<td>1.05 (0.91 to 1.18)</td>
<td>0.52</td>
<td>1.04 (0.87 to 1.26)*</td>
<td>0.65*</td>
</tr>
<tr>
<td>Cows' Milk Fat (%)</td>
<td>0.98 (0.79 to 1.17)</td>
<td>0.87</td>
<td>1.05 (0.84 to 1.31)*</td>
<td>0.66*</td>
</tr>
</tbody>
</table>

*Adjusted for child sex, age in months, household family income, maternal ethnicity, child zBMI score, hours of outdoor freeplay, multivitamin supplementation, vitamin D supplementation and sugar sweetened beverage consumption. Multivitamin supplementation, vitamin D supplementation, maternal ethnicity and sugar sweetened beverage consumption were all measured at exposure, the remaining covariates were measured at time of outcome.

uRR= Unadjusted Relative Risk
aRR = Adjusted Relative Risk

**reported is the relative risk of fracture for each additional cup of milk daily and relative risk of fracture for each 1% increase in milk fat content respectively.
Chapter 4: Overall Discussion

In this thesis, I have attempted to evaluate two relationships: 1) The relationship between cows’ milk volume consumed in early childhood and risk of fracture in later childhood; and 2) The relationship between cows’ milk fat content consumed in early childhood and risk of fracture in later childhood. Finally, I explored the role of milk fat consumed on the relationship between cows’ milk volume and risk of fracture. I identified that neither cows’ milk volume or fat content consumed in early childhood was associated with increased risk of fracture in later childhood. Lastly, the milk fat content consumed did not modify the relationship between milk volume and risk of fracture.

Cows’ milk contains calcium, vitamin D, fat, protein and many other vitamins and minerals that are needed for bone growth and mineralization (9, 17). It has been promoted by the dairy industry, and supported by many organizations including Health Canada, as an important component of a healthy diet for children to optimize bone health (4, 116, 129). Therefore, I hypothesized that a higher volume of cows’ milk or higher cow’s milk fat consumed in early childhood would be protective against fracture in later childhood. To my knowledge, no previous research has prospectively evaluated the association between cows' milk volume or fat and risk of childhood fracture.

Previous studies which have examined the relationship between cows' milk consumption and childhood fracture have had inconsistent findings (8, 12). A systematic review and meta-analysis on early nutrient intakes and childhood fracture identified 2 case-control studies which were of high quality (12, 15, 104). A case-control study by Ma and colleagues from Australia involved 206 cases and 206 controls aged 9-16 years, and found no difference in volume of cows’ milk consumed between fracture cases and controls (104). The other case-control study by Ryan et al. examined 76 fracture cases and 74 controls age 5-9 years from the United States and also found no difference in the mean weekly number of milk servings between cases and controls (12). The authors of this systematic review and meta-analysis concluded that insufficient evidence was available to determine if cows' milk consumption in childhood is protective against childhood fractures (12). A more recent case-control study from the TARGet Kids! group included 137 cases and 343 controls also found no difference in fracture risk among children who consumed less than 2 cups of cows’ milk per day vs children who consumed the recommended 2 cups per
day (55). A systematic review of RTC's did not identify existing clinical trial data on the relationship between cows’ milk and fracture risk but did identify studies which showed that higher dairy consumption among children with low baseline intake improved measures of bone strength including BMC and BMD (18). However, BMC and BMD are proxy measures for fracture risk and may not generalize to increased risk of bone fracture in childhood (16).

Although several studies have found associations between cows' milk avoidance and an increased risk of childhood fracture (12), the underlying cause for milk avoidance may itself be the reason for increased risk of fracture. CMPS and lactose intolerance, which are the two main reasons for milk avoidance in childhood (113), can cause chronic diarrhea which has been hypothesized to interfere with nutrient absorption, including calcium and vitamin D and increase the risk of childhood bone fracture (101).

Sugar-sweetened beverage consumption has also been associated with an increased risk of fracture (103, 104), and some authors have hypothesized that displacement of cows’ milk might mediate this relationship (104). However, several studies which have evaluated this hypothesis did not find that cows’ milk mediated the association between sugar-sweetened beverage consumption and childhood fracture risk (103, 104).

Current North American guidelines recommend that children between 1-3 years of age consume 2 cups of cows' milk daily (4, 109, 116, 130, 131). These recommendations are based on plausible mechanistic evidence using proxy outcome measures for fracture prevention. “Milk is good for your bones” is often touted but (9, 17), to my knowledge, no prospective studies have found that cows’ milk consumption is associated with childhood fracture prevention. Several investigators have speculated on the inconsistency between the evidence about cows’ milk for fracture prevention and industry, health professional and public health recommendations for cows’ milk consumption in childhood (Table 1). One investigator (non-systematically) reviewed the literature and found little evidence to support nutritional guidelines which recommend increasing cows’ milk consumption for promoting bone mineralization (8). Further, some publications based on adult populations have found a paradoxical increased risk of fracture associated with cows’ milk consumption (52, 128). While there may be other benefits of cows’ milk consumption in childhood, such as a convenient and economical source of important macro
nutrients (9) and increased growth (107), these need further study to justify current recommendations.

My thesis is also unique in examining the relationship between cows’ milk fat content consumed in early childhood and fracture risk in later childhood, a relationship that to my knowledge has not previously been reported. Although I found no association between milk volume or milk fat and fracture risk, it is possible cows’ milk fat could have modified the relationship between milk volume and fracture risk. As shown by Vanderhout et al. (51) and others (15, 55, 87), milk fat has an independent relationship with adiposity and an independent relationship with vitamin D (51), both of which have been individually associated with fracture risk. Therefore, it is possible that a relationship between cows’ milk volume and fracture risk was modified by the milk fat the child was consuming at the given volume reported. However, I did not find that cows’ milk fat modified the relationship between cows’ milk volume and risk of fracture.

This thesis has raised a number of unanswered questions which could be addressed through future research. For example, are physiologic effects of calcium and vitamin D, which are contained within cow’s milk, sufficient to justify current cows’ milk recommendations for children? Are measures of bone strength such as BMD and BMC, which have been widely used as proxies for fracture prevention, sufficient to justify existing guidelines without evidence supporting fracture prevention per se? Are other benefits of childhood cows’ milk consumption, such as being a convenient and economical source of potentially limiting nutrients (2), sufficient to justify current recommendations? Is cows’ milk consumption in childhood of such low risk that recommendations to support it are justified without evidence of better health outcomes?

Future research is needed to address these questions and clarify whether cows’ milk plays a role in fracture prevention. I suggest that future research move beyond mechanistic thinking and proxy outcomes and focus on clinical outcomes like child or adolescent fractures. While the time horizon of my study extended to 10 years of age, my observation window may not have been long enough to observe a reduction in fracture risk. Fracture reduction in adolescence, adulthood or the senior years from early childhood cows’ milk consumption remains possible. Studies with a longer time horizon would be helpful in answering these questions. Future research on other
potential benefits of cows’ milk consumption in childhood such as increased growth, and obesity prevention could be studied to inform future cows’ milk recommendations.

Strengths of the research contained within this thesis include *a priori* specified hypotheses based on a thorough review of the literature. The study involved a large, ethnically diverse population of healthy children. TARGet Kids! is the largest childhood cohort in Canada (115). The large sample size (n=2466) combined with detailed clinical data allowed me the opportunity to adjust for a number of potential confounders and to measure the strength of clinically meaningful relationships with a small margin of error. Also, the ethnically diverse TARGet Kids! population made my results potentially applicable to other ethnically diverse populations of children seen in the primary healthcare setting. My hypotheses, that higher milk volume and/or fat content, would be associated with reduced fracture risk was based on a thorough review of the literature and determined *a priori*. The data which I used as well as my statistical analysis plan was pre-specified using a structured data creation plan process. This was important to ensure transparency, to maintain the statistical significance of my p-value cut off (p<0.05), and to ensure that my findings are reproducible (*Appendix A*). The statistical method I chose to use was a modified Poisson regression model (121). Poisson regression is usually applied to count data, however I chose to use this method over standard logistic regression because the preferred effect size measure for evaluating clinical meaning, in prospective studies is relative risk (RR) which Poisson regression can provide (132). The output of logistic regression is an odds ratio (OR). This can be converted to RR but this is tedious, can produce inaccurate confidence intervals and lead to an overestimation of RR, even with a large sample size and rare outcome (133, 134). Therefore, Poisson regression is recommended in this scenario to obtain RR directly, and provide more conservative effect size estimates (122). To further minimize the risk of artificially inflated effect sizes, I chose to use a sandwich estimator, as described by Zou et al (121). It is referred to as a sandwich estimator because the variance is sandwiched between two matrices. This method is used to avoid overestimating relative risk estimates which can arise when Poisson regression is applied to binomial data (121). Following the primary and secondary analysis I completed an exploratory analysis to validate and strengthen my findings. First, I explored a potential effect modification by cows’ milk fat on the relationship between cows’ milk volume and risk of fracture. I analyzed this relationship with the use of a likelihood ratio test. I then tested for non-linearity within the relationship between cow’s milk volume and
fracture as well as the relationship between milk fat content and childhood fracture but did not find evidence of non-linearity. Further I re-ran the primary analysis, including the exposure of cow’s milk volume classified categorically by cups, then and stratified by the currently recommendations (less than 2 cups, 2 cups, more than 2 cups) and found no difference in the association with childhood fracture (Appendix D). Finally, the prospective cohort design which I used allowed me to take into account directionally in the hypothesized relationships. Since cows' milk consumption was measured at 1-3 years of age and fracture was measured at 3-10 years of age the possibility of reverse causality is unlikely.

Limitations of my work include misclassification bias. Misclassification bias occurs when information is recalled inaccurately from the past (135). This may have been a limitation as my exposure, outcome, and covariates were all measured by parent report. As many parents may be aware of the current recommendations and guideless about cows’ milk, my study may have been susceptible to social desirability bias, with parents selecting the answer that is perceived as healthy or correct instead of selecting what happened in reality. However, the questionnaire used to obtain milk consumption data was based on the CCHS, which has been used extensively in similar populations (11). Furthermore, measurement of milk intake and fractures were separated in time by, on average ~4 years, making parental awareness of the study hypotheses between exposure and outcome measurement unlikely. While numerous potential confounders were adjusted for in the statistical models, residual confounding remains a possibility. In other words, confounding may still have occurred through variables that were unmeasured and therefore not accounted for in my statistical models. Although cows' milk is the main dietary source of calcium and vitamin D for most Canadian children (37), we were unable to account for other dietary sources of calcium due to data limitations. For example, data on cheese and yogurt consumption, as well as plant-based sources of calcium such as spinach and almonds, were not available. All Canadian children’s supplements that contain a daily dose of 400 IU (10μg) of vitamin D. Therefore, children who consumed a vitamin D supplement or a multivitamin daily were considered as a yes in supplementing with vitamin D. However, in the analysis I was unable to determine the amount of total vitamin D consumed from all sources. I was also unable to determine the mechanism of fracture as the fracture outcome measure was dichotomized as yes or no by parental report from the question “has your child even broken a bone?” Adjustment for the fracture mechanism could have been helpful in accounting for fracture mechanisms which
may not be sensitive to nutritional factors or preventable through nutrition recommendations, such as automobile accidents (136). Future studies which account for the mechanism of fracture (such as high impact fractures) may be helpful in teasing apart fractures most sensitive to nutritional factors. Although the TARGet Kids! population is ethnically diverse, it may not be representative of all urban children. For example, children included in my study routinely attended primary health care visits, and these children may be different from those who do not attend routine health care visits. While similar to census and other data on Torontonians, some characteristics of my study population such as 68% daily vitamin D supplementation and 55% of household income over $150,000/year may be different from other urban populations. It is possible that no association was seen between cows’ milk consumption and childhood fracture because of this higher income population. I hypothesize that children in higher socioeconomic status families may have access to better nutrition and therefore more fracture resistant bones resulting in a minimal additive effect of cow’s milk on fracture risk. Lastly, children consuming extremely low or high volumes of cows' milk were in infrequent in this population (10% < 1 cup/day and 7% > 4 cups/day); therefore, the power to detect effects in these children may have been low.

Providing parents with an evidence-base for day-to-day nutritional choices such as cows’ milk consumption is important. While childhood fracture risk has been associated with lifestyle factors such as increased physical activity and higher adiposity, I do not believe there is sufficient evidence to support early childhood cows’ milk consumption for the purpose of fracture prevention in later childhood.

**Chapter 5: Conclusion**

In this thesis, I have identified that neither cows’ milk volume or fat content consumed in early childhood were associated with risk of fracture in later childhood. Further, the milk fat content consumed did not modify the relationship between milk volume and risk of fracture. This research may be helpful to parents, healthcare practitioners, and policymakers when considering fracture prevention as a justification for cows’ milk consumption in childhood. Future research is needed to identify whether specific fracture mechanisms may be sensitive to cows’ milk consumption, whether childhood cows’ milk consumption results in fracture prevention in
adolescence or adulthood and whether other potential health benefits might justify current recommendations for cows’ milk consumption in childhood.
## Chapter 6: Appendices

### APPENDIX A: DATA CREATION PLAN

<table>
<thead>
<tr>
<th>Prospective-Cohort Study Dataset Creation Plan</th>
</tr>
</thead>
</table>

**Name of Study**
- The association between milk volume and milk fat consumed at ages 1-3 and the risk of bone fracture from ages 3-10.

**PI and P&B Contacts**
- Dr. Jonathon Maguire
- Riley Allison

**DCP update history**
- Version 1: February 22nd 2017
- Version 2: February 28th 2017
- Version 3: March 2nd 2017
- Version 4: April 27th 2017
- Version 5: May 8th 2017
- Version 6: May 15th 2017
- Version 7: July 10th 2017
- Version 8: September 21st 2017
- Version 9: November 10th 2017

**Short Description of Research Question**
- **Primary:**
  - Is higher volume of milk consumed between the ages of 1-3 years associated with lower fracture risk between ages 3-10 years
  - Is consumption of higher milk fat content at age 1-3 associated with a lower risk of fracture in childhood from ages 3-10.
- **Secondary:**
  - How does the milk fat consumed of milk modify relationship between milk volume and fracture risk?

**List of Datasets Used**
- TARGet Kids! Baseline pre-migration (before September 2011)
- TARGet Kids! Baseline post-migration

### Defining the Cohort

**TARGet Kids! Cohort**
- Children ages 1-10 who attended well-child visits at a primary care paediatrician's or family physician's office. With in this group we are looking at children who drink non- cow's milk and have sustained at least one fracture in their lifetime.

**Exclusions in Cohort (In order)**
- Children with associated health conditions affecting growth (e.g. failure to thrive, cystic fibrosis)
- Children with an chronic condition(s) except for asthma
- Children with severe developmental delay
- Families who are not fluent in English
- Children born premature (children born < 34 weeks gestation)

**Size of Primary Cohort**
- Number of children recruited from December 2008 to September 2016 who filled out the NHQ questionnaire.
### Time Frame Definitions

**Accrual Start/End Dates**  December 2008 to February 2017

### Variable Definitions

<table>
<thead>
<tr>
<th>Main Exposure or Risk Factor</th>
<th>Primary Exposure: Cows milk volume (% as continuous) <em>Taken at the 1-3 year visit.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHQ(22)(Pre-Migration- NHQ #24 &amp; #54) Circle how many 250 ml cups of each drink your child has currently in a typical day. (all fat percentages of cow’s milk)</td>
</tr>
<tr>
<td></td>
<td>The milk group includes the choices: 0 cups, 0.5 cups, 1 cup, 2 cups, 3 cups, 4 cups and 5+ cups.</td>
</tr>
<tr>
<td></td>
<td><strong>Continuous variable [reference values: Cups 0,0.5,1,2,3,4,5+]</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary Exposure: Cows milk fat % consumption (% as continuous) <em>Taken at the 2-3 year visit.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>NHQ(22)(Pre-Migration- NHQ #24 &amp; #54) Circle how many 250 ml cups of each drink your child has currently in a typical day.</td>
</tr>
<tr>
<td>The milk group includes the below choices:</td>
</tr>
<tr>
<td>• Cows Milk</td>
</tr>
<tr>
<td>• Cows Milk 1%</td>
</tr>
<tr>
<td>• Cows Milk 2%</td>
</tr>
<tr>
<td>• Cows Milk Homo (Whole Milk 3%)</td>
</tr>
<tr>
<td>• Children who consume multiple milk-fat %’s, their multiple values will be averaged deriving a % between the reference groups. (i.e a child who is drinking 1% and 2% equally will be given a value of 1.5%)</td>
</tr>
<tr>
<td><strong>Continuous Variable [reference values: 0.1%(skim), 1 %, 2%, 3.25%]</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Variables may include:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Age (years)</td>
</tr>
<tr>
<td></td>
<td>• Sex (M/F)</td>
</tr>
<tr>
<td></td>
<td>• zBMI</td>
</tr>
<tr>
<td></td>
<td>• Socioeconomic Status (based on mean neighbourhood median after-tax household income (from postal code)</td>
</tr>
<tr>
<td></td>
<td>• Maternal Ethnicity (used for possible cultural implications e.g., feeding practices) (Mixed Western, Mixed Western and Non-Western, East and Southeast Asian, Southwest Asian, African and Caribbean)</td>
</tr>
<tr>
<td></td>
<td>• Cows Milk Consumption (Cups/day)</td>
</tr>
<tr>
<td></td>
<td>• Cows Milk Consumption (Fat % Type consumed)</td>
</tr>
<tr>
<td></td>
<td>• Level of Physical Activity (Outdoor free play Minutes/day)</td>
</tr>
<tr>
<td></td>
<td>• Sugar Sweetened Beverage Consumption</td>
</tr>
<tr>
<td></td>
<td>• Multivitamin supplementation</td>
</tr>
<tr>
<td></td>
<td>• Vitamin D supplementation</td>
</tr>
<tr>
<td></td>
<td><strong>Others List</strong></td>
</tr>
<tr>
<td></td>
<td>• Organized Activity/Sports (Hours/weekday) &amp; (Hours/weekend day)</td>
</tr>
<tr>
<td></td>
<td>• Calcium Supplementation</td>
</tr>
</tbody>
</table>
Primary Outcome: Parent reported Fracture(s) *Taken between ages 3-10
- (NHQ #17)(Pre-Migration- NHQ #13 & #37) Has your child ever broken a bone?
- Categorical [Reference Values: Yes or No]

Covariates for primary analysis

1. Age (years)
   Continuous variable

2. Sex
   Categorical variable [reference value: Female ]

3. Family income/ Socioeconomic Status
   Categorical variable (Less than $30,000, $30,000-$79,999, $80,000-$150,000, More) [Reference Value: $30,000-$79,999]

4. Child Vitamin D Supplementation
   (NHQ #18a) (Pre-Migration- NHQ #38) Does your child take any vitamins or supplements regularly? Vitamin D?
   Categorical Variable [reference value:Yes ]
   4b. Amount of Vit D (International Units) Continuous

5. Milk Fat % Consumed
   NHQ(22)(Pre-Migration- NHQ #24 & #54) Circle how many 250 ml cups of each drink your child has currently in a typical day.
   The milk choices include: Skim Milk, 1% milk, 2% milk and Whole Milk
   Continuous Variable [ Reference Values: 0.1,1,2,3.25 ]

5. Child Multivitamin supplementation
   (NHQ #18a) (Pre-Migration- NHQ #38) Does your child take any vitamins or supplements regularly? Multivitamin (with or without iron)?
   Categorical Variable [reference value:Yes ]

6. Child zBMI
   Child weight and Height measured at primary care visit
   BMI calculated using ( weight kg/ height m²) Continuous variable

7. Outdoor Free-Play
   (minutes/day) Continuous variable

8. Child Sugar Sweetened Beverage Consumption
   (NHQ #28)(Pre-Migration- NHQ #24 & #54) Circle how many 250 ml cups of each drink your child has currently in a typical day (Sweetened drinks, Soda or Pop)
   Categorical Variable [reference value: 0 Cups per day, value= No, If >0 cups per day, Value = Yes ]

9. Maternal Ethnicity
   Categorical Variable (European , East Asian, South Asian, South East Asian, Arab, African American, Latin American, and Mixed)
   Categorical Variable [reference value: European]
Analysis Plan

1. Analysis Tables:
   a) Table 1 of N(%) or mean (SD) for baseline characteristics of population
   b) Table 3 of N(%) for study variable by exposures unadjusted
   c) Table 4 of N(%) for study variable by exposures adjusted
   d) Table 5 of N(%) for study variable by exposures adjusted with interaction term

2. Analysis:

Modified Poisson Regression Primary
   * Run unadjusted modified Poisson regression model to test association between volume of cows milk and fracture.
   * Run unadjusted modified Poisson regression model to test association between milk fat % consumed and fracture.
   * Run adjusted modified Poisson regression model to test association between cow’s milk volume and fracture including factors known or suspected to affect their relationship
   * Run adjusted modified Poisson regression model to test association between cow’s milk fat % and fracture including factors known or suspected to affect their relationship
   * Fracture (yes) = Cows Milk Volume + age + Sex + zBMI + Outdoor Free Play + Sugar Sweetened Beverage Consumption + Child Vitamin D Supplement + Maternal Ethnicity + Socio-Economic Status + organized activity
   * Fracture (yes) = Cows Milk fat % + age + Sex + zBMI + Outdoor Free Play + Sugar Sweetened Beverage Consumption + Child Vitamin D Supplement + Maternal Ethnicity + Socio-Economic Status + organized activity
   * Use Variance Inflation Factor to test for multicollinearity in covariates. Remove variables with VIF >5 before performing multiple logistic regression analysis. Most important for exposure variable
   * Run adjusted zero inflated Poisson regression model to test association between volume of cows milk and fracture including factors known or suspected to affect their relationship. As well for the number of fractures as the outcome
   * Validate the fit of the model by using bootstrap and Cross-Validation

Modified Poisson Regression Exploratory
   * Run adjusted zero inflated Poisson regression model to test association between cow’s milk volume and fracture, with cows milk fat % as an interaction term, and factors known or suspected to affect their relationship. Validate the fit of the model by using bootstrap and Cross-Validation
APPENDIX B: CONCEPTUAL MODEL FOR STUDY 1

Primary Exposure:
Volume of Cows Milk Consumed (Cups/Day)
Cows' Milk Fat content consumed (%)

Effect Modifier: Cows' Milk Fat Content Consumed (%)

Primary Outcome: Parental report of Childhood fracture

Confounders
- Age
- zBMI
- Socioeconomic Status
- Maternal Ethnicity
- sugar sweetened beverage consumption
- Calcium Supplementation

Predictors of Outcome
- Sex
- Outdoor Free-play
- Vitamin D Supplementation
- Multivitamin supplementation
APPENDIX C: COWS MILK CONSUMPTION BY FRACTURE YES/NO

Box plot of fracture yes/no by cows' milk consumption
APPENDIX D: PARTICIPANT AGE DISTRIBUTION

Age of Participants at Exposure and Outcome

Age in Months

Age during the exposure window  Age during the outcome window
APPENDIX E: COWS’ MILK CONSUMPTION

Frequency of Cows' Milk Consumption By Cups

Number of participants

Cups of Cows' Milk
APPENDIX F: STUDENT CONTRIBUTIONS

1. Conduced literature review, curated research questions and designed analyses

2. Performed statistical analysis using R version 0.99.903

3. Presented research findings at two conferences

4. Wrote thesis

5. Submitted research article study 1 Milk Volume, Milk Fat and Childhood Fracture Risk for publication: JAMA – Pediatrics
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