Protocol

Effects of Milk or Yogurt Supplementation on Bone Health, Body Composition, and Gut Microbiota in Canadian Young Adults: Protocol for a Three-Arm Parallel-Group Randomized Controlled Trial

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Abstract

Background: Osteoporosis is a major global public health concern. Optimizing peak bone mass during young adulthood is the main preventive strategy for reducing fracture risk later in life. Dairy foods, such as milk, provide nutrients, including calcium and protein, that support bone health, while fermented dairy foods, including yogurt, may confer additional benefits through potential modulation of the gut microbiome. However, the long-term comparative effects of milk and/or yogurt supplementation compared with no dairy supplementation on bone health, body composition, and the gut microbiome in Canadian young adults remain unknown.

Objective: This study aims to evaluate the effects of 2-year supplementation with fermented (yogurt) and nonfermented (milk) dairy products, compared to a habitual diet, on femoral neck bone mineral density (BMD) in Canadian adults aged 19 to 30 years, and to directly compare the effects of milk versus yogurt supplementation. The secondary objectives include assessing changes in BMD at skeletal sites, including the total hip, lumbar spine, and whole body, as well as body composition, hormonal factors, bone turnover markers, and gut microbiota composition and diversity following the intervention.

Methods: The Fermented-Non-Fermented-Dairy trial is a 2-year, prospective, randomized, 3-arm, parallel-group controlled trial. Eligible healthy adults (N=120) aged 19 to 30 years living in Saskatoon, Canada, with calcium intake of <800 mg per day were randomized to one of three arms: (1) milk (375 mL) with habitual diet, (2) yogurt (350 g) with habitual diet, or (3) control (habitual diet). Data are collected at 5 time points: baseline and months 6, 12, 18, and 24. The primary outcome is the femoral neck BMD change. The secondary outcomes include BMD at other skeletal sites (total hip, lumbar spine, and whole body), bone mineral content (total hip, femoral neck, lumbar spine, and whole body), bone structure and geometry, biochemical markers of bone turnover (osteocalcin, bone-specific alkaline phosphatase, C-terminal telopeptide of type 1 collagen, transforming growth factor [TGF]-β1, TGF-β2, TGF-β3, and TGF-β receptor II), bone-related hormonal markers

(parathyroid hormone, 25-hydroxyvitamin D, and serum and salivary insulin-like growth factor 1), anthropometrics, body composition (total fat and lean mass), and gut microbiota.

Results: Recruitment was conducted between July 2024 and July 2025, with an allocation ratio of 1:1:1 for the 3 study arms. Currently, month 12 follow-ups are ongoing. Month 24 assessments will take place from November 2026 to July 2027, followed by data analysis and dissemination of findings.

Conclusions: This study uses a robust methodology and multidimensional outcome assessment to evaluate the effects of dairy supplementation on skeletal and metabolic health in young adults. The findings will contribute to evidence on dairy-based nutritional strategies to optimize long-term bone health in Canadian young adults.

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Introduction

Osteoporosis and related fragility fractures represent a major global public health concern, as they account for 8.9 million fractures globally [1]. In Canada, an estimated 839 per 100,000 individuals aged ≥40 years were affected by osteoporosis in 2024 [2]. The Canadian health care system continues to face significant challenges due to the serious health consequences and economic burden associated with osteoporosis [3]. Although osteoporosis is most commonly diagnosed in older adults, it begins to develop earlier in life [4]. Peak bone mass, reached in early adulthood, strongly influences lifelong skeletal health, and modest increases can reduce fracture risk later in life [5,6]. Therefore, identifying effective nutritional strategies during young adulthood represents an important preventive approach.

The bone modeling and remodeling process, which includes key steps of bone formation and resorption during the phase of growth and development, allows bones to widen and lengthen until they fully mature [7]. The amount of bone mineral acquired during this period is specific to age and sex and reaches its peak approximately 6 years after adolescents attain peak height [8], typically by the end of the second decade or the start of the third decade [9]. More specifically, the total bone mineral content (BMC) of the total body reaches a plateau at 19 years of age in women and 21 years of age in men [9], with accompanying changes in bone geometry affecting bone strength. These bone-building processes are sensitive to environmental factors, including diet, physical activity, and hormonal status [10]. Adequate intake of calcium and high-quality protein plays a central role in supporting bone mineralization and maintaining skeletal integrity [11]. Calcium provides the primary mineral substrate for bone, while dietary protein contributes to bone matrix formation and influences anabolic hormones such as insulin-like growth factor 1 (IGF-1) [12]. Lifestyle factors, including weight-bearing physical activity, further modulate bone strength and structure [13]. Given the dynamic nature of bone metabolism in young adulthood, this life stage presents a critical window for preventive nutritional interventions to promote bone health.

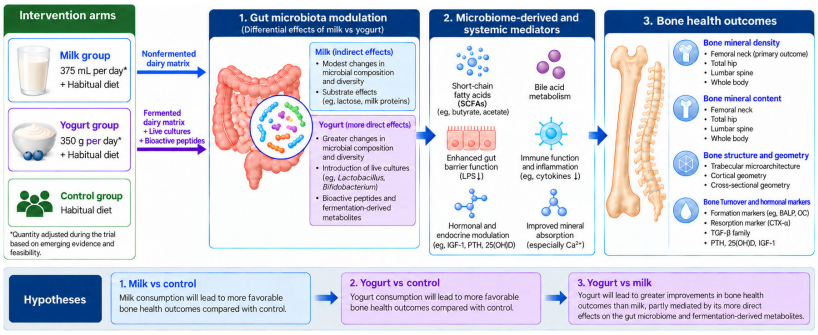
Dairy products are widely recommended as important dietary sources of calcium, protein, and other nutrients, such as phosphorus, magnesium, potassium, and vitamin D through fortification [14]. Dairy protein contains essential amino acids that support muscle mass and may indirectly benefit bone through mechanical loading and endocrine pathways [15]. For Canadians aged 19 to 30 years, the estimated average requirement (EAR) for calcium is 800 mg per day [16], while the recommended dietary allowance is 1000 mg [16]. Data from the Canadian Community Health Survey reveal a declining trend in milk consumption, with average servings decreasing from 1.2 to 0.9 per day between 2004 and 2015, alongside a corresponding reduction in calcium intake [17]. Additionally, revisions to Canada's Food Guide have moved dairy products into the "protein foods" group, potentially reducing the emphasis on dairy intake [18,19]. Observational studies have generally reported positive associations between dairy intake and bone mineral density (BMD) [15,20]. However, such evidence is subject to residual confounding and cannot establish causality [10]. Randomized controlled trials (RCTs) examining dairy supplementation have been conducted, but many have been short in duration, focused on children or older adults, or limited to single outcomes [21-23].

Among dairy products, milk and yogurt are the most commonly consumed [24,25]. An additional gap in the literature concerns differences in the effects of fermented and nonfermented dairy products. Milk and yogurt differ in biological properties. The fermentation process in yogurt reduces lactose content, alters protein structure, and introduces live bacterial cultures that can influence digestion and metabolism [24]. It may also produce bioactive peptides and metabolites with distinct physiological effects [24]. Emerging evidence suggests that fermented dairy consumption may support favorable metabolic and inflammatory profiles compared with nonfermented dairy [26]. However, direct comparisons of milk and yogurt within the context of bone and gut health, particularly in a long-term randomized design in healthy adults, are limited.

The gut microbiome has recently been receiving growing attention as a potential mediator of bone health. This

relationship is often referred to as the gut-bone axis. The intestinal microbiota can influence nutrient absorption, immune function, and systemic inflammation, affecting skeletal health [27]. The conceptual framework linking dairy intake, gut microbiota–mediated pathways, and bone health outcomes is illustrated in Figure 1. Short-chain fatty acids produced through microbial fermentation may enhance calcium absorption and modulate bone remodeling [27]. Probiotic and prebiotic interventions have demonstrated positive effects on bone health in animal models and selected human populations. Both milk and yogurt may influence the gut microbiome through their nutrient composition and dairy matrix; however, yogurt is expected to exert more direct and potentially greater microbiome-modulating effects because of its live cultures and fermentation-derived bioactive compounds [28]. Integrating microbiome assessment into a bone-focused trial may therefore provide insights into mechanisms beyond traditional nutrient-based explanations. Body composition is another key aspect of bone health. Lean mass supports bone density through mechanical loading and hormonal pathways [29]. Dairy protein intake may support muscle mass growth and maintenance, indirectly influencing bone outcomes [15], as it is shown that greater lean mass is associated with higher femoral neck BMD [30]. Conversely, adipose tissue releases inflammatory cytokines that stimulate osteoclast activity and increase bone resorption [31]. A comprehensive assessment of whole-body composition, alongside measuring bone outcomes, allows for a more integrated evaluation of musculoskeletal health.

Figure 1. Conceptual model of the gut-bone axis in the milk and yogurt randomized controlled trial. Long-term consumption of dairy products, particularly fermented dairy products such as yogurt, may modulate gut microbiota composition and metabolic activity, influencing systemic mediators such as short-chain fatty acids (SCFAs), immune function, and endocrine pathways. These mechanisms may, in turn, affect bone health outcomes. This figure was generated with the assistance of an AI-based tool (DALL·E; OpenAI) and refined by the authors. 25(OH)D: 25-hydroxyvitamin D; BALP: bone-specific alkaline phosphatase; CTX-α: α C-terminal telopeptide of type I collagen; IGF-1: insulin-like growth factor 1; LPS: lipopolysaccharide; OC: osteocalcin; PTH: parathyroid hormone; TGF-β: transforming growth factor beta.



Despite growing interest in dairy, bone health, and the microbiome, several limitations characterize the current evidence base. To our knowledge, to date, no food-based clinical trial has comprehensively evaluated the impact of long-term dairy consumption on bone health measured in terms of mineral density, mineral content, shape, structure, and other bone outcomes [32]. Few studies have incorporated advanced imaging techniques such as high-resolution peripheral quantitative computed tomography (HR-pQCT) to evaluate bone microarchitecture. The composition of the oral and gut microbiomes has been explored in the Canadian population but primarily in older adults or individuals with subclinical conditions [33,34]. Evidence supports the efficacy of probiotics in the healthy population [35-37]; however, this association has not yet been investigated among Canadians aged 19 to 30 years. Additionally, limited trials have simultaneously assessed structural bone outcomes, endocrine biomarkers, and gut microbiota composition within the same randomized framework. These gaps highlight the need for a comprehensive and methodologically rigorous trial.

This 3-arm, parallel-group RCT aims to investigate the effects of 2-year daily supplementation of fermented (yogurt) and nonfermented (milk) dairy products or continuation of habitual diet (control) on bone health, body composition, and gut health outcomes in Canadian adults aged 19 to 30 years. The primary objective is to evaluate the impact of daily milk or yogurt supplementation, compared with no dairy supplementation, on femoral neck BMD, a clinically important site, given that femoral neck density is one of the strongest predictors of future fracture risk [38], while the secondary objectives include examining the effects of milk and yogurt supplementation, compared with no dairy supplementation, on (1) BMD and BMC at other skeletal sites, including lumbar spine, total hip, and whole body, as well as bone structure and geometry; (2) body composition outcomes, such as bone-free lean tissue mass, total body fat mass, abdominal fat mass, and percentage of total body fat; (3) hormonal indices, including parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], and growth factors such as serum and salivary IGF-1; (4) markers of bone turnover (osteocalcin, bone-specific alkaline phosphatase [BALP], C-terminal telopeptide of type 1 collagen [CTx-1], transforming growth factor [TGF]-β1, TGF-β2, TGF-β3, and TGF-βRII); and (5) gut microbiota composition, encompassing taxonomic profiles and α- and β-diversity. We also aim to compare the effects of milk versus yogurt supplementation on bone health and body composition outcomes.

We hypothesize that, when compared to the control group, supplementation with milk or yogurt among Canadians aged 19 to 30 years will (1) improve bone health indices, as evidenced by an increase in femoral neck BMD; (2) increase

total lean body mass; and (3) not result in an increase in total fat mass. Given the distinct microbial and bioactive properties of yogurt [39], we further hypothesize that (4) yogurt supplementation, compared with milk supplementation, will result in greater improvements in bone health and body composition outcomes, accompanied by more favorable alterations in gut microbiota composition and diversity.

Methods

Trial Design and Registration

This study, referred to as the Fermented-Non-Fermented-Dairy (FNFD) trial, is a 2-year, prospective, randomized, 3-arm, parallel-group controlled trial designed to evaluate the effectiveness of daily milk and yogurt supplementation compared with a habitual diet (control) in adults aged 19 to 30 years. Participants were randomly allocated in a 1:1:1 ratio to one of three groups: (1) milk group (habitual diet plus milk supplementation), (2) yogurt group (habitual diet plus yogurt supplementation), and (3) control group (habitual diet).

This trial was registered on ClinicalTrials.gov (NCT06265246) on February 20, 2024. This protocol is reported in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2025 checklist for standard protocol items (Checklist 1) [40]. The trial results will be reported following the CONSORT (Consolidated Standards of Reporting Trials) guidelines [41].

Study Setting

The trial is being conducted in Saskatoon, Saskatchewan, Canada. Participant recruitment, data collection, and analyses are taking place at the University of Saskatchewan (USASK) campus in Saskatoon, Saskatchewan.

Sample Size

Sample size estimation was based on the primary outcome, change in femoral neck BMD, using a superiority framework. Calculations were informed by a previously published randomized trial that reported a postintervention femoral neck BMD of 0.831 g/cm^2 with an SD of 0.092 g/cm^2 in the milk intervention group, compared with a BMD of 0.782 g/cm^2 in the control group, corresponding to an absolute difference of 0.049 g/cm^2 [42].

Assuming a 2-sided significance level of 0.05 and 80% power to detect a between-group difference of this magnitude, a minimum of 28 participants per group was required. To account for an anticipated attrition rate of up to 30% [43] over the 2-year intervention period, the target sample size was increased to 40 participants per group. Accordingly, a total sample size of 120 participants was required for the 3-arm trial.

Recruitment Strategy

Participants were recruited on a rolling basis using a multimodal strategy. Recruitment methods included distributing study posters, flyers, and study information cards, as well as announcements through the USASK student portal. Furthermore, recruitment was conducted using targeted social media advertising through Facebook, Instagram, and LinkedIn to reach eligible participants. All recruitment materials included a QR code and web link directing interested individuals to an online screening survey and detailed study information. Additional recruitment support was provided by community organizations and Saskatoon Leisure Clubs.

The trial was publicly listed in national clinical trial registries, including the Canadian clinical trials database, and was featured on the “Be the Cure” platform to enhance visibility and accessibility. Recruitment started in July 2024 and continued until July 2025.

Eligibility Criteria

Eligibility was assessed using a structured screening questionnaire (demographics and brief health history). To assess eligibility with respect to calcium intake, the Automated Self-Administered 24-Hour Dietary Assessment Tool [44] was used during the screening process. Participants were required to meet all inclusion criteria and none of the exclusion criteria listed in Textbox 1.

Textbox 1. Inclusion and exclusion criteria.

Inclusion criteria

- Adults (men and women) aged 19 to 30 years
- Currently residing in Saskatoon or the surrounding area, Saskatchewan, Canada, with a plan to remain in the area for the duration of the study
- Total habitual calcium intake from diet and supplements of <800 mg per day
- Willingness to consume dairy products daily for 24 months if randomized to an intervention group
- Ability to provide written informed consent and comply with all study procedures, including baseline and follow-up assessments over the 2-year study period

Exclusion criteria

- Demographic and reproductive factors
  - Another household member or sibling already enrolled in the trial to maintain independent observations and minimize contamination across study arms
  - Pregnancy, lactation, or intention to become pregnant during the study period
- Medical history and health status

- History of significant bone, endocrine, or metabolic disorders known to affect bone metabolism, including osteoporosis or other bone diseases, hyperparathyroidism or other endocrine disorders, uncontrolled thyroid disease, chronic liver or kidney disease, connective tissue disorders, hypogonadism, or conditions associated with prolonged immobilization
- Major chronic illness or recent medical condition that could pose a risk to the participant or influence study outcomes, including active cancer, malabsorption syndromes, inflammatory bowel disease, uncontrolled diabetes, or chronic respiratory diseases requiring systemic corticosteroids
- Use of medications known to affect bone or calcium metabolism, including chronic corticosteroids, bisphosphonates, calcitonin, anticonvulsants affecting vitamin D metabolism, thiazide diuretics, heparin, or other medications that could confound bone-related outcomes
- Concurrent participation in another intervention study or program likely to affect bone health or gut microbiome outcomes, such as dietary supplementation trials, structured weight loss programs, or exercise intervention studies

- Dietary factors
  - Diagnosed lactose intolerance or dairy-related intolerance severe enough to preclude regular consumption of milk or yogurt
  - Known allergy to cow's milk or dairy proteins

Young adulthood, defined here as ages 19 to 30 years, represents a critical life stage for achieving and consolidating peak bone mass and establishing long-term skeletal health before age-related bone loss begins [45]. Restricting enrollment to individuals with total calcium intake below 800 mg per day was based on the EAR for calcium and was intended to target individuals with suboptimal calcium intake, thereby maximizing the ability to detect the effects of milk and yogurt supplementation on bone outcomes.

Randomization and Sequence Generation

Eligible participants were randomized to 1 of the 3 study arms using the REDCap randomization module [46]. The random allocation sequence was computer generated by the study biostatistician before starting the trial. A permuted block randomization approach was used to maintain approximate balance across study groups over time, with randomly varying block sizes of 6 and 9 that were not disclosed to investigators, research assistants, and participants. Randomization was stratified by sex at birth (male or female) and age group (19-22, 23-26, and 27-30 years), as these factors are known to influence bone outcomes. Separate allocation sequences were generated for each stratum. The allocation ratio was 1:1:1 within each stratum. Randomization occurred once eligibility was confirmed. To enhance transparency and participant engagement, participants initiated the randomization process by activating the randomization function within REDCap under staff supervision.

Allocation Concealment Mechanism

Allocation concealment was ensured through centralized randomization using REDCap. Allocation sequences were stored securely within the REDCap system and were inaccessible to investigators, research staff, and participants before assignment. At the time of randomization, enrolling staff entered the participant's stratification variables into REDCap, after which the system automatically assigned the participant to the next available intervention according to the preprogrammed sequence. Group assignment (milk, yogurt, or control) was revealed at the moment of allocation.

Blinding

Evidence has shown that blinding is often challenging and sometimes not possible in food-based trials [47]. Randomization was conducted in the presence of the participant to allow appropriate provision of intervention products. To minimize potential bias, outcome assessors and laboratory personnel involved in biological sample processing, imaging technicians conducting bone scans, and statisticians performing data analyses are blinded to group allocation. Study groups are coded using neutral labels to maintain blinding during outcome assessment and analysis.

Unblinding

As participants and intervention staff are aware of group assignments, unblinding procedures primarily apply to outcome assessors and analysts. No circumstances requiring emergency unblinding are anticipated, as the interventions involve commonly consumed foods and pose minimal risk. Group identities will remain masked to statisticians until the dataset is locked and all prespecified primary and secondary analyses, including intention-to-treat (ITT) and per-protocol analyses, have been completed.

Study Interventions

Intervention Description

The FNFD study had 2 interventions, namely milk and yogurt. All participants, regardless of group assignment, were instructed to continue their habitual diet throughout the 2-year period of study. Participants randomized to the milk group were instructed to consume 1.5 servings of milk (equivalent to 375 mL) per day, providing approximately 500 mg of calcium daily, and could choose between 1% or 2% milk fat options. Participants randomly assigned to the yogurt group consumed 2 servings of yogurt (equivalent to 350 g) per day, providing approximately 500 mg of calcium daily, and had

the option to choose between plain yogurt with 2% milk fat or flavored yogurt with 3% milk fat.

Intervention products are provided to participants on a weekly or biweekly basis, depending on participant preference and logistical considerations, such as refrigerator storage capacity and travel to the study site. Participants are instructed not to cook or freeze the dairy products to preserve the viability of live bacterial cultures.

Comparator

Participants assigned to the control group continue their habitual diet for the duration of the study without the provision of additional dairy products. No placebo product is used.

Adherence Strategies

Intervention adherence is monitored subjectively using repeated 24-hour dietary recalls and compliance surveys administered through REDCap. Bimonthly compliance surveys assess adherence to the prescribed intervention, frequency and quantity of dairy consumption, and barriers to adherence. When nonadherence is identified, individualized support and dietary counseling are provided to address challenges and promote continued compliance.

Participants experiencing challenges with adherence may book an online consultation with a registered dietitian, who works with participants to identify barriers and provide personalized strategies to support compliance. In addition, intervention-specific recipes on a study-dedicated website are shared to offer creative options for meeting recommended intake.

Concomitant Care

Participants are instructed to maintain their usual dietary patterns and physical activity levels throughout the study period. To minimize potential confounding, participants are discouraged from initiating new dietary supplements containing calcium, vitamin D, or other nutrients relevant to bone or metabolic outcomes during the trial. Use of a standard multivitamin is permitted if the participant was already taking one at baseline. High-dose calcium or vitamin D supplements and other bone-active supplements are not permitted during the study period. Participants are also asked to avoid major changes in habitual diet, such as initiating a vegan diet or a high-protein diet, and to refrain from substantial changes in exercise habits. Medications or therapies required for existing or new health conditions may continue as part of routine medical care. However, initiation of new medications or therapies with potential effects on bone metabolism or related outcomes is recorded, including information on type, dose, and timing, and reviewed by the study investigators. Aside from these stipulations, there are no prohibited concomitant care activities.

Outcomes

Primary Outcome

The primary outcome is the change in femoral neck BMD of the left hip, measured using dual-energy X-ray absorptiometry (DXA) at baseline, month 12, and month 24. BMD is expressed in g/cm^2. Femoral neck BMD was selected as the primary outcome because it is a clinically relevant site for osteoporotic fracture risk [38] and is responsive to nutritional interventions over extended follow-up in young adulthood [48]. Femoral neck BMD values will be interpreted in relation to World Health Organization reference criteria [49] to characterize bone health status.

Secondary Outcomes

BMD and BMC at Additional Skeletal Sites

Secondary bone outcomes include BMD and BMC at the total hip, lumbar spine from L1 to L4, and whole body, assessed using DXA [50] at baseline, month 12, and month 24. These outcomes will determine whether intervention effects observed at the femoral neck are also present at other clinically relevant skeletal sites. Whole-body scans will also provide total body BMC.

Bone Microarchitecture and Geometry

Microarchitectural and geometric parameters will be assessed using HR-pQCT [51] at the distal radius and distal tibia. Measurements will be performed at baseline and month 24 to evaluate trabecular and cortical bone structure beyond areal BMD. Additionally, hip structural analysis will be conducted.

Body Composition

Body composition outcomes include total body fat mass, total body lean mass, percent body fat, and regional adiposity, including trunk and limb compartments [52]. These outcomes will be assessed using whole-body DXA at baseline, month 12, and month 24.

Gut Microbiome Composition and Diversity

Gut microbiome outcomes include changes in taxonomic composition and α- and β-diversity metrics, with particular focus on genera associated with dairy fermentation, such as *Lactobacillus* and *Bifidobacterium* [53]. Stool samples will be collected at baseline, month 12, and month 24.

Exploratory and Mechanistic Outcomes

Hormonal Indices

Hormonal markers related to bone metabolism will be assessed at baseline, month 12, and month 24, and include PTH, serum 25(OH)D, and IGF-1 [54]. PTH and serum 25(OH)D will be measured in blood samples, and IGF-1 will be measured in both serum and saliva.

Biochemical Markers of Bone Turnover

Markers of bone formation and resorption [55] will be assessed in fasting blood samples collected at baseline, month 12, and month 24. These include osteocalcin, BALP, CTx-1, TGF-β1, TGF-β2, TGF-β3, and TGF-βRII.

Participant Timeline

The schedule of enrollment, interventions, and assessments is summarized in Figure 2. Eligible participants provided written informed consent and were randomized to 1 of the 3 study arms. After baseline data collection, participants started consuming the intervention and will continue for 24 months. Major study outcome assessments were conducted as planned at baseline, with month 12 assessments currently ongoing. Month 24 follow-up assessments will be carried out as scheduled as participants complete 2 years in the study.

Figure 2. Schedule of enrollment, interventions, and assessments. *In person; **remotely; ***at home. DXA: dual-energy X-ray absorptiometry; HR-pQCT: high-resolution peripheral quantitative computed tomography.

Study Period	Enrollment	Allocation	Post-allocation (Intervention period)				
			Month 0	*Month 6*	*Month 12*	*Month 18*	*Month 24*
Enrollment							
Eligibility screen	X						
Informed consent	X						
Randomization		X					
Interventions							
Control (habitual diet)			X →	→	→	→	→
Milk group			X →	→	→	→	→
Yogurt group			X →	→	→	→	→
Assessments							
Sociodemographic data			X*				
24-h dietary recalls (×3)			X*	X**	X*	X**	X*
Dairy Food Frequency			X*	X**	X*	X**	X*
Physical activity			X*	X**	X*	X**	X*
Bone-specific physical activity					X*		X*
Household Food security			X*		X*		X*
Sun safety behaviors			X*		X*		X*
Smoking and alcohol consumption				X**		X**	
Individual Typology Angle (spectrophotometer)					X*		X*
DXA scan			X*		X*		X*
HR-pQCT			X*				X*
Blood sample (fasting)			X*		X*		X*
Saliva sample			X*		X*		X*
Stool sample			X***		X***		X***
Waist circumference			X*	X**	X*	X**	X*
Height and weight			X*		X*		X*
Bi-monthly compliance survey			Bi-monthly**				

Data Collection Methods

Data for the FNFD trial are collected using REDCap, a secure, web-based data capture platform hosted on the USASK domain [46]. REDCap provides structured electronic data capture with built-in validation, audit trails to track data entry and modifications, and secure data export to common statistical software packages [56]. Figure 2 outlines the variables and instruments used in data collection.

Sociodemographic and Socioeconomic Characteristics

Sociodemographic and socioeconomic data are collected at baseline using items adapted from the Canadian Community Health Survey (CCHS) [57]. Variables include age, sex at birth, household composition, number of individuals and children in the household, country of birth, province or territory of birth for Canadian-born participants, immigration status and length of residence in Canada, Indigenous identity, racial or cultural background, languages spoken, employment status, household income sources, total household income category, supplemental health insurance coverage, and highest level of education attained.

Dietary Intake

Dietary intake is assessed every 6 months at 5 time points: baseline, month 6, month 12, month 18, and month 24. At each time point, three 24-hour dietary recalls are collected,

scheduled approximately 10 to 15 days apart. Two recalls reflect weekday intake, and at least one recall captures intake on a weekend day. Nutrient intakes are averaged across the 3 recalls to estimate usual dietary intake during each assessment period. If completion of 3 recalls is not feasible, a minimum of 2 recalls is required. A dairy-specific food frequency questionnaire is also administered.

Baseline, month 12, and month 24 dietary recalls are performed in person, while the month 6 and month 18 recalls are conducted via telephone. Research assistants receive standardized training in dietary data collection and use the multiple-pass method [58], a validated approach for improving recall accuracy. Dietary data are entered into REDCap and subsequently coded and analyzed using Elizabeth Stewart Hands and Associates (ESHA) Food Processor software. The dairy food frequency questionnaire captures information on frequency and type of dairy consumption, use of dairy alternatives, and self-reported gastrointestinal discomfort associated with dairy intake.

Physical Activity

Physical activity is assessed every 6 months at 5 time points: baseline, month 6, month 12, month 18, and month 24. General physical activity is measured using the Physical Activity Adult Questionnaire (PAAQ), which captures transportation, recreational, occupational, and household physical activity performed during the previous 7 days. Questionnaire responses are used to estimate minutes per week spent in activities of varying intensity. The PAAQ has been implemented in national population surveys, including the Canadian Health Measures Survey [59] (CHMS; 2014-2015 cycle) and the CCHS (2015 [60] and 2016 [57]).

To capture bone-relevant mechanical loading, a bone-specific physical activity questionnaire is also administered [61]. This instrument records both current and historical physical activity. Ground reaction force–derived loading values are applied to calculate bone loading scores, and raw questionnaire responses are converted into composite scores reflecting total bone-relevant physical activity [61].

Household Food Security

Household food security is assessed using the Household Food Security Survey, adapted for the Canadian context and used in Canadian Community Health National surveys [62]. The module consists of 18 items that assess food insecurity related to financial constraints experienced over the previous 12 months. The questionnaire is self-administered in person at baseline, month 12, and month 24. Responses are used to classify households into standard food security categories. Households with no affirmative responses are classified as food secure. Food-insecure households are categorized as marginally food insecure, moderately food insecure, or severely food insecure based on the number of affirmative responses, following established scoring guidelines.

Sun Safety Behavior

Sun exposure and sun protection behaviors are assessed at 3 time points (ie, baseline, month 12, and month 24) using the Sun Safety Behavior Questionnaire, which was implemented in the CCHS [63] in 2010. The questionnaire captures information on time spent outdoors in the sun and use of protective behaviors, including wearing protective clothing, seeking shade, and sunscreen use during sun exposure. Sun safety behavior is measured to account for individual differences in UV exposure, a major determinant of serum 25(OH)D and downstream bone metabolism [64].

Smoking and Alcohol Consumption

Smoking behavior and alcohol consumption are assessed using standardized questionnaire modules [65]. The smoking module captures information on current and former smoking status, while the alcohol module assesses alcohol consumption patterns over the previous 12 months. These questionnaires are administered remotely via REDCap at month 6 and month 18.

Skin Pigmentation

Skin pigmentation is assessed using the Individual Typology Angle (ITA) measured by a calibrated spectrophotometer. Measurements are conducted in person at month 12 and month 24. Assessments are obtained at three anatomical sites: (1) the interosseous muscle area of the right hand between the thumb and index finger, (2) the inner surface of the upper right arm, and (3) the center of the forehead. At each site, 3 consecutive readings are collected. The spectrophotometer records color parameters in the CIE Lab* color space, including L* representing lightness, a* representing the red-green axis, and b* representing the blue-yellow axis. These parameters are derived from light reflectance at multiple wavelengths and are used to calculate the ITA as an objective measure of skin color and pigmentation over time. ITA strongly influences cutaneous vitamin D synthesis and therefore affects bone metabolism [66].

Bone and Body Composition Measurements

Dual-Energy X-Ray Absorptiometry

Bone and body composition measurements are assessed at baseline, month 12, and month 24 using DXA (Hologic Inc) at the USASK bone research laboratory. BMD and BMC are measured at the femoral neck, total hip, lumbar spine, and whole body. Hip structural analysis is conducted using DXA-derived images to assess structural characteristics of the proximal femur, including the narrow neck region, the intertrochanteric region along the bisector of the neck-shaft angle, and the femoral shaft [67].

Body composition outcomes include bone-free lean tissue mass, total body fat mass, abdominal fat mass, and percent total body fat, all derived from whole-body DXA scans. During scanning, participants are positioned supine with arms pronated and feet dorsiflexed according to standardized protocols [68]. All scans are performed by certified densitometry technicians following manufacturer-recommended positioning and acquisition procedures. The effective radiation dose associated with DXA scanning is minimal, with high measurement precision [69]. Precision error for the

femoral neck BMD measured through the DXA scan is 1.9% [70].

High-Resolution Peripheral Quantitative Computed Tomography

Bone microarchitecture, geometry, and volumetric BMD (vBMD) are assessed using HR-pQCT [51] at baseline and month 24. Scans are conducted at the distal radius and distal tibia at the Laboratory for Imaging Muscle and Bone Structure within the Physical Activity Complex at USASK. HR-pQCT is a noninvasive imaging technique that provides a high-resolution 3D assessment of trabecular and cortical bone structure with low radiation exposure [51]. The technique enables measurement of vBMD, expressed in milligrams of hydroxyapatite per cubic centimeter, and allows differentiation of bone geometry, thickness, and tissue mineral density that cannot be distinguished using DXA. Outcome measures include total, trabecular, and cortical vBMD, as well as structural and microarchitectural parameters derived from cross-sectional images. vBMD measurements are not influenced by bone size or body composition [51] and permit differentiation between changes in bone geometry and tissue mineral density, which cannot be distinguished using areal BMD measures. Reported precision errors for HR-pQCT range from approximately 0.13% to 2.5% for total vBMD and from approximately 0.5% to 4% for trabecular and cortical compartments [71].

Biochemical Measurements

Biochemical measurements are conducted at 3 time points: baseline, month 12, and month 24. Fasting blood samples are collected from each participant between 9 AM and 10 AM to minimize diurnal variation in hormonal and metabolic markers. Participants are instructed to fast overnight for at least 12 hours before blood collection and to refrain from engaging in strenuous physical activity for 48 hours before the visit. Blood collection is performed by trained personnel using standardized phlebotomy procedures. A total of approximately 20.5 mL of blood is collected at each time point, consisting of one 4.5 mL lithium heparin plasma separating tube and four 4 mL serum separating tubes. Plasma and serum samples are labeled with anonymized study IDs to ensure participant confidentiality and maintain blinding during laboratory analyses.

Plasma samples are processed immediately following collection. Tubes are gently inverted, centrifuged according to standardized laboratory protocols, aliquoted, and temporarily stored at 2 °C to 8 °C for up to 3 days before analysis. Plasma is used for the assessment of PTH and serum 25(OH)D concentrations. PTH is measured using the COBAS Elecsys PTH (1-84) assay [72], and serum 25(OH)D is measured using the Elecsys Vitamin D total III assay [73]. Both assays are performed on the Cobas e801 analyzer (Roche Diagnostics, Switzerland) following manufacturer specifications and established quality control procedures.

Serum samples are allowed to clot, centrifuged, and aliquoted into ten 400 µL threaded-cap cryovials per participant. These are stored at –80 °C until batch analysis. Serum aliquots will be used for the analysis of IGF-1 and markers of bone formation and resorption, such as osteocalcin, BALP, and CTx-1, as well as regulatory cytokines and growth factors, including TGF-β1, TGF-β2, TGF-β3, and TGF-βRII.

Biomarker concentrations are determined using validated and commercially available enzyme-linked immunosorbent assays (ELISAs). All assays are conducted in accordance with manufacturer protocols, with appropriate calibration standards, quality controls, and triplicate measurements included to ensure accuracy, precision, and reproducibility of results. Batch analysis is performed to minimize interassay variability, and laboratory personnel remain blinded to participant group allocation throughout biochemical analyses.

Salivary IGF-1

Salivary IGF-1 is assessed as a noninvasive biomarker of growth factor activity at 3 time points: baseline, month 12, and month 24. Saliva samples are collected using the Super•SAL Universal Saliva Collection Kit [74] (Oasis Diagnostics Corporation) following the manufacturer's standardized collection protocol. Participants are instructed to refrain from eating, drinking beverages other than water, chewing gum, smoking, or brushing their teeth for at least 30 minutes before sample collection to minimize contamination and variability. Saliva collection is conducted under the supervision of study personnel to ensure protocol adherence. Once collected, samples are handled according to the procedures provided by the collaborating laboratory, including centrifugation to remove particulate matter and aliquoting into prelabeled cryovials.

Aliquoted saliva samples are stored at –80 °C until analysis and are batch processed to reduce interassay variability. Salivary IGF-1 concentrations are quantitatively determined using a solid-phase sandwich ELISA [75]. Specifically, the DuoSet ELISA Development Kit for human IGF-1 from R&D Systems is used for quantitative determination following the manufacturer's protocol.

Gut Microbiota

Gut microbiota composition is assessed using stool samples collected at 3 time points: baseline, month 12, and month 24. Participants collect stool samples using the OMNIgene stool collection kits (DNA Genotek), following standardized instructions provided by the research team to ensure consistency and sample integrity. Upon receipt at the study laboratory, stool samples are processed at room temperature. Samples are aliquoted into 2 threaded cap cryovials containing approximately 250 to 500 µL each and are immediately stored at –80 °C until further processing. This approach minimizes degradation and preserves microbial DNA for downstream analyses.

Total genomic DNA is extracted from fecal material using the QIAamp PowerFecal Pro DNA Kit [76], following the manufacturer's validated extraction protocol (QIAGEN N.V.). This kit is specifically designed to efficiently lyse

microbial cells and remove inhibitors commonly present in stool samples. Following extraction, DNA yield and purity are assessed using a NanoDrop spectrophotometer [77] (Thermo Fisher Scientific) to ensure adequate concentration and acceptable purity ratios before sequencing. Extracted DNA samples are kept at –20 °C until further analysis.

Microbial community composition is characterized by sequencing the bacterial 16S ribosomal RNA gene, targeting the V3-V4 hypervariable regions, using the Illumina MiSeq platform (Illumina Inc) [78]. Sequencing data are used to assess overall gut microbial composition and diversity, including α- and β-diversity metrics. Relative abundance of bacterial taxa is determined, with specific focus on genera commonly associated with dairy fermentation and probiotic activity, including *Lactobacillus* and *Bifidobacterium*.

Sequencing data are processed using the standardized Quantitative Insights into Microbial Ecology (QIIME 2) bioinformatics pipelines [79] to generate taxonomic profiles and diversity measures. These microbiome outcomes are used to evaluate longitudinal changes in gut microbial composition in response to milk and yogurt supplementation using the Analysis of Compositions of Microbiomes with Bias Correction 2 [80] R package and to explore correlations and potential mechanistic links between dairy intake, gut microbiota, and bone and metabolic outcomes.

Anthropometrics

Height and body weight are measured using standardized procedures. Height is measured to the nearest 0.1 cm using a wall-mounted stadiometer, with participants standing barefoot, upright, and aligned with the stadiometer, heels together, and arms at their sides. Participants are instructed to inhale and maintain an erect posture during measurement. Three height measurements are obtained, and the average value is used for analysis. Body weight is measured to the nearest 0.01 kg using a calibrated digital floor scale (SECA 877), with participants wearing light clothing and no shoes or jewelry. Three weight measurements are obtained, and the average value is used for analysis.

Central adiposity is assessed using waist circumference measurements collected every 6 months. Waist circumference is measured at the midpoint between the lowest rib and the iliac crest and recorded to the nearest 0.1 cm. Three measurements are obtained at each assessment, and the average value is used for analysis. Measurements are obtained in person at baseline, month 12, and month 24. At month 6 and month 18, waist circumference is self-reported by participants using standardized measurement instructions provided by the research team.

Personnel Training

All study personnel involved in data collection and intervention delivery received standardized training before study initiation to ensure consistency, protocol adherence, and data quality. Training included instruction on informed consent procedures, administration of study questionnaires and assessment tools, use and calibration of equipment, and accurate data entry and management within the REDCap system. Blood sample collection is performed exclusively by qualified phlebotomists with appropriate training and certification. Bone imaging assessments, including DXA and HR-pQCT scans, are conducted by trained and certified technicians following standardized protocols. All research personnel involved in handling and distributing intervention products are trained in food safety procedures, including cold chain management, safe storage, and hygienic handling practices, to ensure intervention quality and participant safety from product receipt through delivery for consumption.

Data Management

Data management is conducted in accordance with Good Clinical Practice principles and applicable confidentiality and data protection regulations.

Data Capture and Entry

Most study data are captured and stored using REDCap, a secure, web-based electronic database hosted on encrypted servers at USASK. Upon enrollment, each participant is assigned a unique numeric study identifier, which is used on all electronic case report forms, questionnaires, and biological samples. Anthropometric and clinical measurements are transcribed into REDCap immediately following collection. Imaging results from DXA and HR-pQCT scans and laboratory results for PTH and serum 25(OH)D are entered into REDCap after verification of participant identification and visit time point.

Data Quality Assurance

Multiple procedures are implemented to ensure high data quality. All study personnel follow a detailed procedures manual that outlines standardized protocols for each measurement. During initial baseline data collection, staff work in pairs under supervision to ensure protocol adherence. REDCap is programmed with validation rules, including range checks and required fields, to minimize data entry errors and missing values.

Equipment used for data collection, including scales, stadiometers, DXA systems, and spectrophotometers, is calibrated regularly according to manufacturer recommendations. Anthropometric measurements are collected in triplicate, with average values used for analysis. To minimize interrater variability, the same research staff members perform specific measurements whenever possible throughout the study. DXA quality assurance includes periodic phantom spine scans to monitor machine stability. DXA outcome values are manually entered into REDCap and independently cross-checked by a second research assistant. HR-pQCT scan quality is assessed through visual inspection for motion artifacts before inclusion in analyses.

Dietary data quality is supported through the collection of three 24-hour recalls at each assessment period. A senior team member reviews culturally specific foods entered into the ESHA Food Processor software. Exported nutrient data are screened for implausible values, and potential entry errors are

corrected following verification. Biological sample quality is ensured through strict adherence to standardized protocols for sample collection, handling, transportation, storage, and analysis. For stool samples, extracted DNA purity and concentration are assessed using spectrophotometry, and absorbance ratios and curves are reviewed before downstream analyses [77].

Data Storage and Security

All electronic data are stored on password-protected, encrypted university servers with daily automated backups managed by institutional information technology services. Access to REDCap is role-based and restricted to authorized study personnel through individual university credentials. REDCap audit trails automatically record all data creation, modification, and export activities. User privileges within REDCap are assigned according to study role, and access is revoked when personnel leave the study. As an additional precaution, deidentified datasets are periodically exported and securely stored in the principal investigator's (PI's) institutional data storage environment. Non-REDCap-based study documents are managed using secure institutional file-sharing platforms with version control. The master linkage file connecting participant identifiers to personal information is stored separately in a secure location and is accessible only to the PI and study coordinator. Physical documents, including signed consent forms and hard copies of imaging or laboratory reports, are stored in locked filing cabinets within secured research offices. Biological samples are labeled with study identifiers and time points only and stored in designated freezers.

Data Sharing

Deidentified individual participant data, along with associated data dictionaries and statistical code, will be made available following publication of the primary trial results. Data will be shared through a secure institutional data repository under a formal data sharing agreement to support secondary analyses while protecting participant confidentiality. Trial results, including null or negative findings, will also be reported in the public clinical trial registry in accordance with reporting requirements.

Statistical Methods

Statistical Analysis Plan

All statistical analyses will be conducted according to a prespecified analysis plan and will primarily follow the ITT principle. Under the ITT framework, participants will be analyzed in the groups to which they were originally randomized, regardless of adherence to the intervention or completion of follow-up, provided that outcome data are available. This approach preserves the benefits of randomization and minimizes bias due to differential attrition or noncompliance. The overall analytical workflow consists of 3 main phases: data cleaning and processing, descriptive analyses, and advanced statistical modeling.

Data Cleaning and Processing

Data cleaning procedures include verification of data accuracy, identification of outliers, assessment of missing data patterns, and evaluation of variable distributions. For quantitative variables, outliers will be identified using distribution-based criteria, including values falling beyond 3 SDs from the mean or outside 1.5 times the IQR in boxplot analysis. Derived variables, such as BMI, will be created as needed. Missing data will be addressed using maximum likelihood with the ignorable missingness method within mixed effects models, which accommodate incomplete observations under the assumption that data are missing at random. Data entry inconsistencies and implausible values will be reviewed and corrected following verification against source documents.

Descriptive Analyses

Descriptive analyses will be used to characterize participant demographics, baseline characteristics, and outcome distributions across study groups and time points. Categorical variables will be summarized as frequencies and percentages, and continuous variables will be summarized as means and SDs for normally distributed data or medians and IQRs for nonnormally distributed data. Normality will be assessed using graphical methods and statistical tests, including the Shapiro-Wilk or Kolmogorov-Smirnov tests, as appropriate. Data transformations may be applied when necessary to meet modeling assumptions. Baseline group comparisons will be conducted using ANOVA for continuous variables and chi-square or Fisher exact tests for categorical variables.

Inferential and Modeling Analyses

The primary outcome, change in femoral neck BMD, and secondary continuous outcomes will be analyzed using linear mixed effects models to account for the repeated-measures structure of the data. In accordance with the study hypotheses, the primary comparisons will evaluate changes in outcomes between the milk supplementation and control groups and between the yogurt supplementation and control groups. In addition, changes in outcomes will be compared between the milk and yogurt supplementation groups. Time of measurement, study group, and the interaction between time and group will be included as fixed effects to assess differential changes over time between each intervention and control group. Prespecified pairwise contrasts from the fitted models will be used to directly test the hypothesized differences between the milk and control groups, between the yogurt and control groups, and between the milk and yogurt groups. Participant-specific random effects will be included to account for within-subject correlation across repeated measurements. Specifically, a participant-specific random intercept will be included to account for the correlation among repeated measurements obtained from the same participant over the 24-month study period and to allow for between-participant variability in outcome levels. Participant-specific random effects will represent the repeated-measures structure of the data and will not refer to the numerous baseline characteristics collected. Random intercept models

will be fitted initially, with random slopes added where appropriate based on model fit and convergence. Model assumptions will be evaluated using residual diagnostics. For outcomes that do not meet linear model assumptions, alternative modeling strategies, including generalized linear mixed models, will be considered.

Potential confounding variables will be identified a priori using directed acyclic graphs developed for each major outcome [81]. Covariates identified as confounders, including relevant biological and lifestyle factors, may be included in adjusted models. Both unadjusted and adjusted models will be reported where appropriate. For categorical outcomes, mixed-effects logistic regression models or other appropriate generalized models will be used. Results will be presented as estimated effects with corresponding CIs and *P* values.

Secondary and Sensitivity Analyses

In addition to ITT analyses, per-protocol analyses may be conducted for the primary outcome as sensitivity analyses. The per-protocol population will be defined as participants without any major protocol violations. These analyses will provide insight into intervention effects under conditions of high compliance. All participants who withdraw after randomization but contribute partial data will be included in the ITT analyses using all available observations. Sensitivity analyses may be conducted to explore the robustness of findings in relation to different assumptions regarding missing data or model specification.

Statistical Software and Blinding

All analyses will be conducted using the R statistical computing environment [82] using appropriate and validated packages. The study statistician will remain blinded to group allocation, with intervention groups coded using neutral labels, until completion of the primary and secondary analyses and finalization of the analytical dataset. Statistical significance will be evaluated using 2-sided tests with an α level of .05, unless otherwise specified. Effect estimates will be reported with corresponding CIs to facilitate interpretation of both statistical and clinical relevance.

Monitoring

Data Monitoring and Oversight

Given the minimal risk nature of this food-based intervention trial, an independent data monitoring committee was not established. Instead, internal monitoring procedures are implemented. Trial oversight is provided by the PI and a physician coinvestigator, who together function as the study safety monitoring group. Additionally, team members from the Agriculture and Agri-Food Canada (AAFC) oversee the study progress. This approach was reviewed and approved by the relevant research ethics boards (REBs). Study data are reviewed at regular intervals to monitor enrollment progress, participant retention, adherence to the intervention, and any reported adverse events. Safety information is also included in annual reports submitted to the REB at USASK and AAFC.

Interim Analyses

No formal interim analyses with statistical stopping rules are planned, as the interventions involve commonly consumed dairy products and pose minimal anticipated risk. A descriptive interim analysis is planned once all participants have completed their 12-month assessment. Due to staggered recruitment, this is expected to occur when the trial reaches approximately 18 months of follow-up.

Trial Monitoring and Audit

Day-to-day trial conduct is monitored internally by the PI and senior research staff. The PI conducts periodic site visits during data collection to ensure adherence to the study protocol. Senior team members perform spot audits of selected data collection stations and observe research team interactions with participants to ensure compliance with standardized procedures. Data entry accuracy is verified through random checks of REDCap entries against source documents, including imaging and laboratory reports. When deviations or errors are identified, corrective actions are implemented, which may include retraining of personnel, workflow modifications, or updates to documentation procedures. An independent auditor from the USASK clinical trials support unit may conduct a monitoring visit to assess compliance with Good Clinical Practice requirements. No external monitoring organization is otherwise engaged, given the single-site design and low-risk nature of the study.

Harms and Adverse Events

Although the trial is considered minimal risk, potential harms are actively monitored. An adverse event is defined as any untoward medical occurrence experienced by a participant during the study, regardless of whether it is considered related to the intervention. Expected adverse events include mild gastrointestinal symptoms, such as bloating, diarrhea, constipation, or changes in appetite or weight. Unexpected adverse events may include allergic reactions or other unanticipated health issues. Serious adverse events are defined according to standard criteria, including events that result in death, are life-threatening, require hospitalization, or result in significant disability. Such events are considered highly unlikely in this nutrition trial but will be documented and reported if they occur.

Assessment and Management of Harms

Participants are instructed to report any symptoms or health concerns throughout the study. Tolerance and side effects are monitored in follow-up visits and compliance surveys. Reported adverse events are recorded in a secure REDCap log, including information on severity, duration, and perceived relationship to the intervention, and are reviewed by the study physician. Mild and transient symptoms are managed with dietary counseling by the study registered dietitian. Persistent or moderate symptoms may prompt temporary intervention adjustment, such as dose reduction or brief interruption.

In the unlikely event of a serious or unexpected adverse event, the event will be reported promptly to the appropriate REBs and sponsor. An independent physician not otherwise involved in the trial may be consulted to advise on participant safety and trial continuation. Significant safety concerns would prompt consideration of protocol modification or trial suspension. No trial-specific insurance is provided; participants access standard health care services as needed. The research team facilitates appropriate referrals and follow-up care in the event of study-related harm.

Protocol Amendments

Several protocol amendments were implemented after trial initiation to improve feasibility, participant adherence, and study retention. All amendments were reviewed and approved by the Biomedical REB at USASK and by AAFC before implementation. The trial registry was updated accordingly.

First, during the early initiation of the trial, higher-than-anticipated attrition was observed in the yogurt group, and participant feedback indicated challenges in tolerating the initial daily dose of 350 g of yogurt. To reduce attrition and improve adherence to the intervention, an amendment was made to the original protocol with respect to the quantity of intervention products. The daily dose for yogurt was reduced from 350 to 250 g. To preserve comparability between intervention arms and ensure equivalent total dairy exposure, the quantity of milk was also reduced from 375 to 250 mL. This modification was applied uniformly to all participants after completion of 6 months on the original dose, such that from month 7 onward, all participants received the revised dosage. Second, the eligibility criterion related to habitual calcium intake was revised. The original upper limit of 800 mg per day was increased to 1500 mg per day. Our screening data indicated that a substantial proportion of otherwise eligible young adults exceeded the initial threshold. Furthermore, in summer-fall 2026, an additional 42 participants with calcium intakes below 800 mg per day were recruited to ensure an adequate sample size of participants with low calcium intake. Third, the anticipated attrition rate used in the sample size calculation was increased from 15% to 30% to reflect observed retention patterns in this 2-year intervention. As a result, the total target sample size increased from 99 to 120 participants, corresponding to 40 participants per group. Where applicable, participants were informed of relevant changes, and consent addenda were administered through REDCap. Participants retained the right to withdraw without penalty following any protocol modification.

Ethical Considerations

Research Ethics Approval

This study is conducted in accordance with recognized ethical standards for human research. Ethical approval has been obtained from the Biomedical REB at USASK (Bio-4648), the Saskatchewan Health Authority, and AAFC (2024-F-010). Annual continuing ethics approvals are obtained from all relevant REBs until study completion. The trial is conducted in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2) [83] and the principles of the Declaration of Helsinki [84]. This manuscript reflects the study protocol version 8, approved by the USASK REB on March 15, 2026.

Informed Consent

Written informed consent is obtained from all participants before enrollment. Eligible participants receive a detailed electronic consent form via REDCap describing the study purpose, procedures, time commitment, potential risks and benefits, confidentiality, and voluntary nature of participation. Participants are given the opportunity to ask additional questions and receive a copy of their signed consent form for their records. Participants are also invited to complete an optional photo or video release form for knowledge dissemination activities.

Confidentiality

Participant confidentiality is strictly maintained. Each participant is assigned a unique study identifier, which is used on all study records and biological samples. No directly identifiable information is included in analytical datasets. Biological samples are labeled with study identifiers only, and microbiome sequencing does not generate identifiable human genetic data. Electronic data are stored securely, and physical documents are kept in locked cabinets within secure research offices. Study communications are conducted through a dedicated study email account accessible only to authorized personnel. Study findings are reported in aggregate form only, with no identifying information included in publications or presentations. Study records are retained for at least 5 years following study completion and then destroyed in accordance with institutional policies. All investigators sign confidentiality and nondisclosure agreements.

Compensation

Participants will receive an honorarium of CAD $360 (US $257.10) in e-gift cards of their choice in recognition of their time and effort. Compensation will be provided at 4 time points: CAD $30 (US $21.42) at 6 months, CAD $100 (US $71.42) at in-person visit 2 (12 months), CAD $30 (US $21.42) at 18 months, and CAD $200 (US $142.83) at in-person visit 3 (24 months). This schedule is intended to acknowledge continued participation and support participant retention.

Withdrawal

Participants may withdraw from the study at any time without consequence. Withdrawal date and reason, if provided, are documented in REDCap. Participants may request that their data collected to date be destroyed and excluded from analyses.

Ancillary and Posttrial Care

Given the minimal risk nature of this dietary intervention trial, no ancillary or posttrial care is planned. Participants experiencing discomfort related to dairy consumption are

advised to discontinue the intervention and seek routine medical care as needed.

Patient and Public Involvement

Participants were not involved in the design of the study. However, participant feedback is collected during the trial to improve study operations, such as intervention distribution logistics. Exit feedback from participants who withdraw is used to inform future research design and implementation.

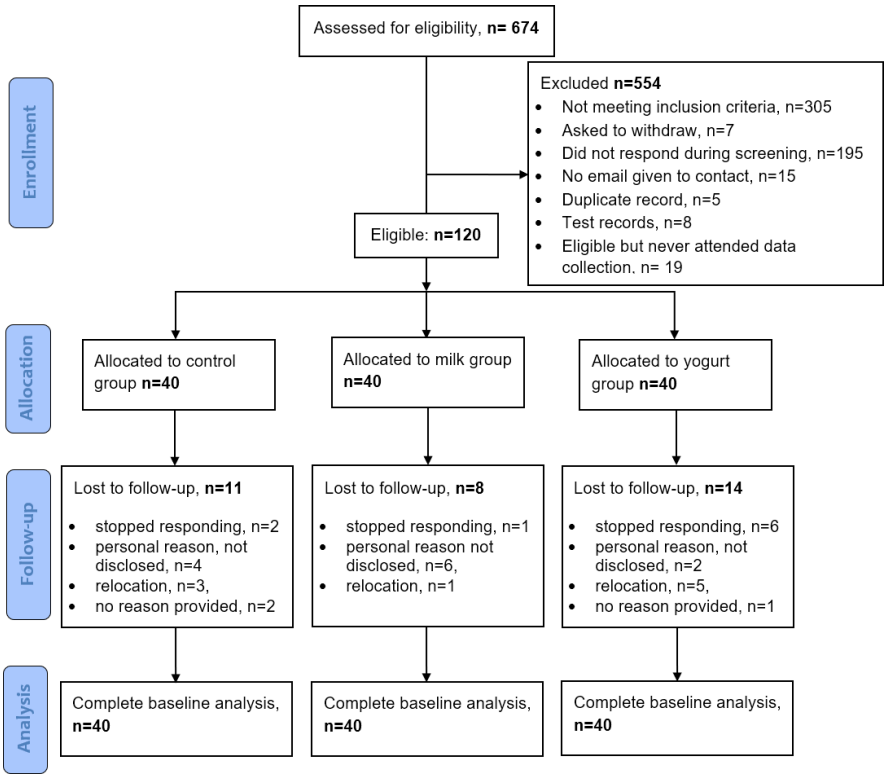
Dissemination of Findings

Study findings will be disseminated to scientific, clinical, and public audiences. Primary and secondary outcomes will be submitted for publication in peer-reviewed journals, with reporting guided by CONSORT and SPIRIT standards. Findings will also be presented at relevant scientific conferences. Participants who indicate interest will receive a lay summary of results following study completion, and a public seminar or webinar will be organized to share findings with the local community and stakeholders. Summary results will be posted on ClinicalTrials.gov within 1 year of study completion, and communication with health professionals, policy stakeholders, and media outlets will be undertaken where appropriate to support knowledge translation and public health relevance.

Results

A total of 120 participants were recruited between July 2024 and July 2025. Participants were randomized to 1 of the 3 study arms in a 1:1:1 ratio. Figure 3 illustrates the CONSORT flow diagram reflecting the current progress in recruitment and data collection. All participants had completed their baseline measurements, and approximately 80% of the baseline data analyses had been completed. Analysis of all baseline outcome measures, including bone formation and resorption biomarkers and stool samples, is pending completion. All month 6 follow-up measurements had been completed at the time of protocol submission. Additionally, the month 12 data collection was ongoing, with more than 40 participants having completed their month 12 follow-up assessments.

Figure 3. CONSORT (Consolidated Standards of Reporting Trials) 2010 flow diagram for the randomized controlled trial as of May 7, 2026.



The FNFD study is currently being conducted under version 8.0 of the protocol. All the changes made to the earlier version of this protocol were subjected to approval from the institutional REB before implementing and updating it in the trial registry. Final (month 24) assessment will start in November 2026 and will be completed in July 2027. By the end of 2027, data analyses will be carried out following the relevant protocols. Aggregated results of the trial will be disseminated through peer-reviewed publications. This will also include reporting in the public trial registry.

Discussion

The FNFD trial is among the first long-term RCTs to integrate dietary intervention, advanced skeletal assessment, and gut microbiome profiling within a single framework. By directly comparing fermented and nonfermented dairy

products, the study is uniquely positioned to examine whether microbiome-related mechanisms contribute to differential effects on bone health, thereby advancing understanding of the gut-bone axis in a healthy young adult population. This integrative approach moves beyond traditional nutrient-based models of bone health toward a systems-level understanding that incorporates host-microbiome interactions. Ensuring skeletal health through gut-microbiome–mediated intervention with dairy products during this period has positive implications for reducing the future likelihood of osteoporosis and fragility risks [6].

This study has several strengths, such as a strong design and long-term duration. The randomized nature of the study with 3 study arms reflects internal validity through reduced selection bias, which enables us to draw a meaningful comparison between intervention arms and the control arm. The long follow-up duration will allow for observation of significant changes in bone architecture and strength, which short-term interventions fail to capture [85]. Using food-based intervention (milk and yogurt) will also improve ecological validity due to cultural acceptance, affordability, and feasibility for long-term adherence compared to a supplement-based long-term study. ITT analysis in this study will ensure methodological rigor. Moreover, this study assesses bone health comprehensively. The assessments complement each other and include bone scans such as DXA and HR-pQCT, hormonal indices (PTH, serum 25(OH)D, and IGF-1), and biochemical indices, including markers of bone formation and resorption. This will provide a multidimensional understanding of skeletal health as it changes over the study duration. Gut microbiome analysis alongside bone health outcomes will provide unique insights into the gut-bone axis. Temporal changes in bone health within and between participants will be explored as the measurements are recorded at 3 major time points, spaced 12 months apart. Data will also be collected on diet, use of supplements, physical activity, smoking and alcohol consumption, and food insecurity, which enables us to adjust for confounding variables in statistical modeling. The study statistician will be blinded to the allocation of participants to the arms to prevent analysis bias. All instruments will be operated using standard protocols to ensure internal validity. The study results will be generalizable to a larger healthy adult Canadian population.

Certain foreseen challenges may exist in this study. Participant attrition is a main concern due to possible relocation or inability to adhere to the intervention protocol. This may introduce attrition bias and may reduce statistical power if loss to follow-up varies across the arms. Moreover, controlling habitual diet, calcium intake from other sources, such as supplements in the control group, sunlight exposure, or physical activity, will not be possible. Although the average will be calculated from the 3 dietary recalls at each time point, there is a possibility of recall bias. Missed visits resulting in missing data may also affect analyses.

The study addresses an existing gap in the literature and will contribute to impactful evidence on the bone and gut health of young Canadian adults. It is expected to generate unique findings in the field of human nutrition and gut microbiome research. The results of this study may drive dietary recommendations for the healthy young adult population in Canada with respect to bone health, supporting nonpharmacological preventive measures for osteoporosis, and guiding nutrition policies. As the intervention is food-based, the trial may provide evidence on whether regular, long-term consumption of calcium-rich dairy products can produce a clinically meaningful benefit over time. This will translate into public health nutrition policies and community-based nutrition programs that aim to improve bone health through locally available and culturally acceptable foods such as milk and yogurt.

In conclusion, this study combines robust methodology with multidimensional outcome assessment to evaluate the impact of milk and yogurt supplementation on skeletal and metabolic health in young adults. The findings are anticipated to contribute to existing evidence on dairy-based nutritional strategies to optimize long-term bone health among young Canadian adults.

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We would like to acknowledge the Dairy Farmers of Canada, Agriculture and Agri-Food Canada, Mitacs Canada, and Vatanparast Nutritional Epidemiology Laboratory for their support of this study.

Generative AI Use

The authors declare the use of generative AI (GenAI) in the research and writing process. According to the GAIDeT (Generative AI Delegation Taxonomy; 2025), the following tasks were delegated to GenAI tools under full human supervision: visualization, proofreading, and editing. GenAI tools used were DALL·E and GPT-5.6 Sol. (OpenAI). Responsibility for the final manuscript lies entirely with the authors. GenAI tools are not listed as authors and do not bear responsibility for the final outcomes. OpenAI DALL·E and GPT-5.6 Sol were used to assist in developing the conceptual framework figure through iterative prompting of the intervention, study design, mechanisms of action, and outcomes. The final figure was refined by the authors. GPT-5.6 was also used for language editing. All content was reviewed and finalized by the authors.

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Authors' Contributions

HV, together with all coauthors, conceptualized and designed the study. HV led the overall preparation of the manuscript, with SAU and MM-V preparing the original draft. SAU, MM-V, and M Shafiee revised the manuscript based on the critical feedback from HV. All authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

None declared.

Checklist 1

SPIRIT checklist.
[DOCX File (Microsoft Word File), 35 KB-Checklist 1]

Peer Review Report 1

Peer review report by the Dairy Farmers of Canada, Sustainable Canadian Agricultural Partnership (SCAP).
[PDF File (Adobe File), 238 KB-Peer Review Report 1]

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Abbreviations

25(OH)D: 25-hydroxyvitamin D
AAFC: Agriculture and Agri-Food Canada
BALP: bone-specific alkaline phosphatase
BMC: bone mineral content
BMD: bone mineral density
CCHS: Canadian Community Health Survey
CHMS: Canadian Health Measures Survey
CONSORT: Consolidated Standards of Reporting Trials
CTx-1: C-terminal telopeptide of type 1 collagen
DXA: dual-energy X-ray absorptiometry
EAR: estimated average requirement
ELISA: enzyme-linked immunosorbent assay
ESHA: Elizabeth Stewart Hands and Associates
FNFD: Fermented-Non-Fermented-Dairy
HR-pQCT: high-resolution peripheral quantitative computed tomography
IGF-1: insulin-like growth factor 1
ITA: Individual Typology Angle
ITT: intention-to-treat
PAAQ: Physical Activity Adult Questionnaire
PI: principal investigator
PTH: parathyroid hormone
RCT: randomized controlled trial
REB: research ethics board
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials
TCPS 2: Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans
TGF: transforming growth factor
USASK: University of Saskatchewan
vBMD: volumetric bone mineral density

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