Supplemental and dietary calcium intakes in postmenopausal women: a series of studies on vascular and bone health

Angel M. Ong

School of Human Nutrition

McGill University, Montreal, Quebec, Canada

May 2020

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

I would like to dedicate this thesis to my beloved mother,

A strong woman who loves with all her heart,

The foundation of my accomplishments

Abstract

Background: Adequate calcium intake is essential for optimal bone health throughout the lifespan. The current Recommended Dietary Allowance (RDA) for calcium for women >50 years is 1200 mg/day and major sources of dietary calcium in the North American diet consist of milk and milk products. However, national surveys have demonstrated a shortfall of calcium in the diet of postmenopausal women, with an average intake of 800 mg/day. Supplemental calcium is therefore often recommended, but controversy over its safety and association with increased risk of cardiovascular events has caused a clinical equipoise. Inconsistent results have been reported in meta-analyses and large cohort studies, and the mechanism that underlies the speculated detrimental effects of supplemental calcium on vascular health remains unknown. Although calcium intake from dietary sources has not been linked with adverse cardiovascular events, recent reports have raised uncertainty as to whether greater milk intake associates with higher all-cause and cardiovascular-related mortality rates. Inflammation plays a role in the pathogenesis of vascular damage, but there is limited evidence from randomized clinical trials in healthy postmenopausal women. Thus, the objectives of this thesis were to: (1) determine the feasibility of conducting a 12-month randomized controlled trial to compare the effect of supplemental versus dietary calcium on markers of vascular health in postmenopausal women; (2) develop and evaluate the relative validity of a semi-quantitative food frequency questionnaire (FFQ) for postmenopausal women; (3) investigate the association of non-fermented and fermented milk products on bone health indicators in postmenopausal women; and (4) investigate the differential effects of supplemental versus dietary calcium on selected inflammatory markers, bone health biomarkers, and body composition outcomes over 12 months.

Design: Study 1 was a pilot randomized trial where postmenopausal women consumed calcium at the level of the RDA, from dietary sources alone (1200 mg/day [CaDiet]) or combined with supplemental calcium (750 mg/day of calcium carbonate with 450 mg/day of dietary calcium [CaSuppl]) over 12 months, with vascular measurements, vascular and bone health biomarkers, anthropometry, and dietary intakes measured. Study 2 was a validation study of a calcium-focused FFQ. Study 3 was a systematic review and meta-analysis of randomized controlled trials, prospective cohort and case-control studies of fermented milk products on bone health outcomes in postmenopausal women. Study 4 was a 12-month randomized controlled trial of similar designs to Study 1, with the addition of a control arm and additional biochemical measures including plasma interleukin-6, leptin, adiponectin, and serum sclerostin.

Results: Results from Study 1 (n=9) showed good compliance to study interventions in both intervention groups (±20% of target total calcium intake, pill count ≥80%). The CaSuppl group maintained a significantly lower average dietary calcium intake than the CaDiet group throughout the trial (453±187 mg/day *versus* 1241±319 mg/day, P<0.001). There was no differential effect on vascular or bone health biomarkers between groups and no adverse effect was reported. Study 2 (n=108) demonstrated the relative reproducibility (r_s=0.72, P<0.001) and validity (κ_W = 0.42) of the 51-item calcium-focused FFQ in estimating usual dietary calcium intake in postmenopausal women. Study 3 included 3 randomized controlled trials, 3 prospective cohort studies and 3 case-control studies in the systematic review, which examined the relationship between individual milk products on hip fracture risk (n=4), osteoporosis (n=2), and bone turnover markers (n=3). The meta-analysis of 3 studies on the association between yogurt intake and hip fracture risk showed a reduced risk (RR 0.76, 95% CI 0.63-0.92), but no association was found in the meta-analysis of 2 studies on the association between cheese intake

and hip fracture risk (RR 0.89, 95% CI 0.73-1.10). The overall quality of evidence was very low. Results from Study 4 (*n*=106) showed that neither of the interventions had an effect on inflammatory markers compared with the control arm at 12-months. However, a significant decrease in C-terminal telopeptide of type 1 collagen in both intervention arms was observed at 12-months. A decrease in bone-specific alkaline phosphatase was found in the CaSuppl arm at the end of the trial. Body composition did not differ among trial arms, but there was a statistically significant increase in body mass index (26.1±4.4 *versus* 25.8±4.2 kg/m^2, *P*=0.04), and percent body fat (35.4±6.3 *versus* 34.5±6.2%, *P*=0.03) in the CaDiet arm at 12-months from baseline.

Conclusion: These results suggest that calcium intake at the level of the RDA, from either dietary sources alone or predominantly from supplemental sources, does not promote systemic inflammation in healthy postmenopausal women. Significant decreases in bone turnover markers following 12-month interventions indicate that the two sources of calcium have similar beneficial skeletal actions in vitamin D sufficient postmenopausal women. These studies contribute evidence to the safety of both dietary and supplemental sources of calcium in healthy postmenopausal women.

Résumé

Contexte: Un apport adéquat en calcium est essentiel pour une santé osseuse optimale tout au long de la vie. L'apport nutritionnel recommandé (ANR) en calcium pour les femmes >50 ans est de 1 200 mg/jour et les principales sources de calcium alimentaire dans le régime nord-américain consistent de lait et produits laitiers. Cependant, des enquêtes nationales ont démontré une carence en calcium dans l'alimentation des femmes ménopausées ayant un apport moyen de 800 mg/jour. La prise d'un supplément de calcium est donc souvent recommandée, mais la controverse sur son innocuité et son association avec un risque accru d'événements cardiovasculaires a provoqué une équipoise clinique. Des résultats divergents ont été rapportés dans plusieurs méta-analyses et grandes études de cohortes, et le mécanisme qui sous-tend les effets néfastes des suppléments de calcium sur la santé vasculaire reste inconnu. Bien que l'apport en calcium provenant de sources alimentaires n'ait pas été lié à la survenue d'événements cardiovasculaires indésirables, quelques rapports récents ont soulevé une incertitude associant une plus grande consommation de lait à un taux de mortalité toutes causes confondues et cardiovasculaire plus élevé. L'inflammation joue un rôle dans la pathogenèse des lésions vasculaires, mais les preuves issues d'essais cliniques randomisés chez les femmes ménopausées en bonne santé sont limitées. Ainsi, les objectifs de cette thèse étaient les suivants: (1) déterminer la faisabilité de mener un essai contrôlé randomisé de 12 mois pour comparer l'effet du calcium sous forme de suppléments par rapport au calcium alimentaire sur les marqueurs de la santé vasculaire chez les femmes ménopausées; (2) élaborer et évaluer la validité relative d'un questionnaire de fréquence alimentaire (QFA) semi-quantitatif sur l'apport en calcium chez les femmes ménopausées; (3) étudier l'association entre les produits laitiers non fermentés et fermentés et les indicateurs de santé osseuse chez les femmes ménopausées; et (4)

étudier les effets différentiels du calcium sous forme de suppléments par rapport au calcium de sources alimentaires sur certains biomarqueurs inflammatoires et de la santé osseuse et les indicateurs de la composition corporelle au cours de 12 mois.

Méthodologie: La première étude (Étude 1) était un essai clinique randomisé pilote où les femmes ménopausées consommaient du calcium au niveau de l'ANR, à partir de sources alimentaires seulement (1 200 mg/jour [CaDiet]) ou combinées avec des suppléments de calcium (750 mg/jour de carbonate de calcium avec 450 mg/jour de calcium alimentaire [CaSuppl]) pendant 12 mois. La rigidité artérielle, la pression artérielle, certains biomarqueurs de la santé vasculaire et osseuse, des mesures anthropométriques et les apports alimentaires ont été mesurés. La deuxième étude (Étude 2) était une étude de validation d'un QFA axé sur le calcium. La troisième étude (Étude 3) était une revue systématique de la littérature et méta-analyse d'essais contrôlés randomisés, d'études de cohorte prospectives et de cas-témoins sur l'association des produits laitiers fermentés sur les paramètres de santé osseuse chez les femmes ménopausées. La quatrième étude (Étude 4) était un essai clinique contrôlé randomisé de 12 mois avec une méthodologie similaire à l'Étude 1, avec l'ajout d'un groupe témoin et des mesures biochimiques supplémentaires, y compris l'interleukine-6, la leptine, l'adiponectine et la sclérostine.

Résultats: Les résultats de l'Étude 1 (n=9) ont démontré une bonne adhérence aux interventions de l'étude dans les deux groupes (±20% de l'apport cible total en calcium; ≥80% nombre de comprimé). Le groupe CaSuppl a maintenu un apport moyen de calcium alimentaire significativement plus faible que le groupe CaDiet tout au long de l'essai (453±187 mg/jour *versus* 1241±319 mg/jour, P<0,001). Il n'y avait aucun effet différentiel sur les biomarqueurs de la santé vasculaire ou osseuse entre les groupes et aucun effet indésirable n'a été signalé. L'Étude 2 (n=108) a démontré la reproductibilité (r_s=0,72, P<0,001) et la validité relative

(κw=0,42) du QFA de 51 items axés sur le calcium dans l'estimation de l'apport habituel en calcium alimentaire chez les femmes ménopausées. L'Étude 3 comprenait 3 essais contrôlés randomisés, 3 études de cohorte prospectives et 3 études de cas-témoins dans la revue systématique, qui ont examiné la relation entre les produits laitiers individuels sur le risque de fracture de la hanche (*n*=4), l'ostéoporose (*n*=2) et les marqueurs biochimiques du remodelage osseux (*n*=3). La méta-analyse de 3 études sur l'association entre la consommation de yogourt et le risque de fracture de la hanche a démontré un risque réduit (RR 0,76, IC à 95% 0,63-0,92), mais aucune association n'a été identifiée dans la méta-analyse de 2 études sur l'association entre la consommation de fromage et le risque de fracture de la hanche (RR 0,89, IC à 95% 0,73-1,10). La qualité globale des preuves était très faible. Les résultats de l'Étude 4 (*n*=106) ont démontré qu'aucune des interventions n'avait d'effet sur les marqueurs inflammatoires par rapport au groupe témoin à 12 mois. Cependant, une diminution significative du télopeptide C-terminal de liaison du collagène de type 1 dans les deux groupes d'intervention a été observée à 12 mois. Une diminution de la phosphatase alcaline osseuse a été observée dans le groupe CaSuppl à la fin de l'essai. La composition corporelle ne différait pas entre les groupes, mais il y avait une augmentation statistiquement significative de l'indice de masse corporelle (26,1±4,4 *versus* 25,8±4,2 kg/m^2, *P*=0,04) et du pourcentage de graisse corporelle (35,4±6,3 *versus* 34,5±6,2%, *P*=0,03) dans le groupe CaDiet à 12 mois par rapport à l'état initial.

Conclusion: Ces résultats suggèrent qu'un apport en calcium provenant des sources alimentaires ou principalement de suppléments équivalent à l'ANR ne favorise pas l'inflammation systémique chez les femmes ménopausées en bonne santé. Une diminution significative des marqueurs biochimiques du remodelage osseux suite à une intervention de 12 mois indiquent que les deux sources de calcium ont des actions squelettiques bénéfiques similaires chez les femmes

ménopausées qui ont un taux suffisant de vitamine D. Ces études apportent des preuves de l'innocuité des sources alimentaires et supplémentaires de calcium chez les femmes ménopausées en bonne santé.

Statement of Support

This work was funded by the Canadian Institutes of Health Research and the Dairy Farmers of Canada. The Research Institute of the McGill University Health Centre provided the facilities to conduct the clinical trial. The PhD Candidate was financially supported by the Research Institute of McGill University Health Centre Studentship and Fellowship Competition (2015) and the operating grant of the clinical trial (Canadian Institutes of Health Research) (2014-2019).

Preface and Advancement of Scholarly Knowledge

This doctoral dissertation is based on the Calcium Study, a randomized controlled trial conducted in 2014-2018, that aimed to investigate the effect of supplemental calcium as compared to dietary calcium on surrogate markers of vascular health in healthy postmenopausal women. No prior prospective research was conducted to estimate the effect of supplemental calcium on vascular endpoints as the primary outcome. Study 1 tested the feasibility of 12-month dietary modifications to meet the RDA from dietary sources alone (1200 mg) or predominantly from supplemental sources (750 mg calcium carbonate + 450 mg dietary). A calcium-focused food frequency questionnaire, which included a comprehensive list of commonly consumed calcium-rich foods in Montreal, Quebec, was developed and validated in postmenopausal women in Study 2. In view of the emerging evidence suggesting differential health benefits from individual milk products, Study 3 examined the relationship between fermented milk products and bone health indicators in postmenopausal women by searching the literature systematically. In line with evidence demonstrating an inverse relationship between fermented milk products and cardiovascular disease risk, the meta-analysis showed a protective association between yogurt consumption and hip fracture risk. These findings support the hypothesis that individual milk products are nutritionally distinct and highlight the importance to examine the health effects of individual milk products separately in investigational studies. Study 4 examined the effect of dietary calcium and supplemental calcium interventions on selected inflammatory markers and markers of bone metabolism. Results of the 12-month trial demonstrated a lack of effect on markers associated with increased risk of vascular calcification from either dietary calcium or supplemental calcium. Cross-sectional analyses of baseline and 12-month data also did not show an association between total milk product nor individual milk product intake with the selected

inflammatory markers. Results of this thesis provide high-level evidence to challenge the concept that supplemental calcium increases cardiovascular risks, and supports current clinical guidelines regarding the safety of calcium intake, from the diet and supplements below the Tolerable Upper Intake Level of 2000 mg/day, for osteoporosis prevention.

Published research articles in peer-reviewed journals

- **Ong AM**, Weiler HA, Wall M, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. Feasibility of a clinical trial to assess the effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women. *Br J Nutr*. 2016 Jul; 116(1):104-14 (Chapter 3)
- **Ong AM**, Weiler HA, Whiting SJ, Wall M, Goltzman D, Daskalopoulou SS, Morin SN. A 51-item calcium-focused food frequency questionnaire is a reliable tool to assess dietary calcium intake in postmenopausal women. *Nutr Res*. 2017 Jul; 43:33-42 (Chapter 4)
- **Ong AM**, Kang K, Weiler HA, Morin SN. Fermented milk products and bone health in postmenopausal women: a systematic review of randomized controlled trials, prospective cohorts and case-control studies. *Adv Nutr.* 2020;11(2):251-65 (Chapter 5)

Abstracts and presentations

- Morin SN, **Ong AM**, Weiler HA, Wall M, Berger C, Das S, Cheung AM, Rahme E, Goltzman D, Daskalopoulou SS. A randomized clinical trial on the effect of dietary calcium intake as compared to calcium supplement on vascular health in postmenopausal women. American Society for Bone and Mineral Research Annual Meeting, September 20-23, 2019, Orlando, Florida, USA. (Oral – Awarded the Most Outstanding Clinical Abstract Award)

- **Ong AM**, Kang K, Weiler HA, Morin SN. Association between fermented milk product intake and bone health indicators in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials, prospective cohorts and case-control studies. Metabolic Disorders and Complications Program Annual Research Day 2019, March 29, 2019, Montreal, Quebec. (Oral)
- **Ong AM**, Kang K, Morin SN, Weiler HA. Association between fermented milk product intake and bone health in postmenopausal women: a systematic review. American Society for Bone and Mineral Research Annual Meeting, September 28 – October 1, 2018, Montreal, Quebec, Canada. (Poster)
- **Ong AM**, Kang K, Morin SN, Weiler HA. Systematic review protocol on the effect of fermented milk products on bone health in postmenopausal women. Metabolic Disorders and Complications Program Annual Research Day 2018, March 29, 2018, Montreal, Quebec. (Poster)
- **Ong AM**, Das S, Weiler HA, Wall M, Daskalopoulou SS, Goltzman D, Morin SN. Relationship between dietary calcium intake and biomarkers of bone and vascular health in healthy postmenopausal women. American Society for Bone and Mineral Research Annual Meeting, September 8-11, 2017, Denver, Colorado, USA. (Poster)
- **Ong AM**, Weiler HA, Wall M, Daskalopoulou SS, Goltzman D, Morin SN. Relationship between usual milk product intake, whole-body adiposity, bone biomarkers and serum lipids in healthy postmenopausal women. 2017 International Workshop on Musculoskeletal and Neuronal Interactions, October 6-8, 2017, Montreal, Quebec, Canada. (Poster)

- **Ong AM**, Weiler HA, Wall M, Daskalopoulou SS, Goltzman D, Morin SN. Association between dietary calcium intake, dairy product consumption and body composition indices in healthy postmenopausal women: a cross-sectional analysis. Experimental Biology Annual Meeting, April 22-26, 2017, Chicago, Illinois, USA. (Poster)
- **Ong AM**, Weiler HA, Wall M, Daskalopoulou SS, Goltzman D, Morin SN. Association between dietary calcium intake, dairy product consumption and body composition indices in healthy postmenopausal women: a cross-sectional analysis. Metabolic Disorders and Complications Program Annual Research Day 2017, March 31, 2017, Montreal, Quebec. (Poster – Awarded second place in poster competition)
- Das S, **Ong AM**, Gomez YH, Gorgui J, Wall M, Goltzman D, Morin SN, Daskalopoulou SS. Dietary calcium intake: does it affect vascular health? Canadian Hypertension Congress, October 19-21, 2016, Montreal, Quebec. (Poster)
- **Ong AM**, Das S, Daskalopoulou SS, Weiler HA, Wall M, Cheung AM, Rahme E, Whiting SJ, Goltzman D, Morin SN. Dietary calcium intake and vascular markers in healthy postmenopausal women. American Society for Bone and Mineral Research Annual Meeting, September 16-19, 2016, Atlanta, Georgia, USA. (Poster)
- Das S**,** Goltzman D, **Ong AM**, Gomez YH, Gorgui J, Wall M, Morin SN, Daskalopoulou SS. Dietary calcium intake and cardiovascular health: is there a relationship? American Society for Bone and Mineral Research Annual Meeting, September 16-19, 2016, Atlanta, Georgia, USA. (Poster)
- Das S, Goltzman D, **Ong AM**, Gomez YH, Gorgui J, Wall M, Morin SN, Daskalopoulou SS. Dietary calcium intake and cardiovascular health: is there a relationship? Ninth

Fellows Forum on Metabolic Bone Diseases, September 15, 2016, Atlanta, Georgia, USA. (Poster)

- Das S**,** Goltzman D, **Ong AM**, Gomez YH, Gorgui J, Wall M, Morin SN, Daskalopoulou SS. Dietary calcium intake and cardiovascular health: is there a relationship? McGill University Endocrinology Retreat, June 2, 2016, Montreal, Quebec. (Poster)
- **Ong AM**, Das S, Daskalopoulou SS, Weiler HA, Wall M, Cheung AM, Rahme E, Whiting SJ, Goltzman D, Morin SN. Dietary calcium intake and vascular markers in healthy postmenopausal women. Research Institute of the McGill University Health Centre Experimental Therapeutics and Metabolism Research Day, May 12, 2016, Montreal, Quebec. (Poster)
- Das S**,** Goltzman D, **Ong AM**, Gomez YH, Gorgui J, Wall M, Morin SN, Daskalopoulou SS. Dietary calcium intake and cardiovascular health: is there a relationship? Research Institute of the McGill University Health Centre Experimental Therapeutics and Metabolism Research Day, May 12, 2016, Montreal, Quebec. (Poster)
- **Ong AM**, Morin SN, Wall M, Goltzman D, Weiler HA. Tobacco use associates with inadequate dietary calcium intake: a cross-sectional study of women living in Montreal. Canadian Nutrition Society Annual Conference, May 5-7, 2016, Gatineau, Quebec. (Poster)
- **Ong AM**, Weiler H, Whiting SJ, Wall M, Hamilton-Leavitt ER, Patel P, Kureishy S, Goltzman D, Daskalopoulou SS, Morin SN. Development and validation of a food frequency questionnaire for estimating dietary calcium intake in postmenopausal women in Montreal, Canada. Réseau de recherche en santé buccodentaire et osseuse (RSBO) Scientific Day, March 11, 2016, Montreal, Quebec. (Poster)

- Das S, Gomez YH, Gorgui J, **Ong AM**, Wall M, Morin SN, Daskalopoulou SS. Dietary intake of calcium associated with surrogate markers of cardiovascular disease in healthy postmenopausal women? Canadian Hypertension Congress, October 22-24, 2015, Toronto, Ontario. (Poster)
- **Ong AM**, Weiler H, Whiting SJ, Wall M, Hamilton-Leavitt ER, Patel P, Kureishy S, Goltzman D, Daskalopoulou SS, Morin SN. Development and validation of a food frequency questionnaire for estimating dietary calcium intake in Canadian postmenopausal women. 9th International Symposium on Nutritional Aspects of Osteoporosis, June 17-20, 2015, Montreal, Quebec. (Poster)
- **Ong AM**, Morin SN, Wall M, Goltzman D, Weiler HA. Dietary calcium intake in postmenopausal women in Montreal, Quebec. Dietitians of Canada National Conference: Canadian Foundation of Dietetic Research, June 5, 2015, Quebec City, Quebec. (Abstract)
- Das S, Gorgui J, Gomez YH, **Ong AM**, Wall M, Morin SN, Daskalopoulou SS. Association of dietary calcium and vitamin D intake with surrogate markers of cardiovascular disease in healthy postmenopausal women. McGill University, McGill Cardiovascular Research Day, May 21, 2015, Montreal, Quebec. (Poster)
- Das S, Gorgui J, Gomez YH, **Ong AM**, Wall M, Morin SN, Daskalopoulou SS. Association of dietary calcium and vitamin D intake with surrogate markers of cardiovascular disease in healthy postmenopausal women. 15th Annual McGill Biomedical Graduate Conference, March 19, 2015, Montreal, Quebec. (Poster)
- **Ong AM**, Weiler H, Wall M, Hamilton-Levitt ER, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. The effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women: results of a 12-month pilot

clinical trial. 15th Annual McGill Biomedical Graduate Conference, March 19, 2015, Montreal, Quebec, Canada. (Poster)

- **Ong AM**, Weiler H, Wall M, Hamilton-Levitt ER, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. The effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women: results of a 12-month pilot clinical trial. Eighth Fellows Forum on Metabolic Bone Diseases, September 10-11, 2014, Houston, Texas, USA. (Poster)
- **Ong AM**, Weiler H, Wall M, Hamilton-Levitt ER, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. The effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women: results of a 12-month pilot clinical trial. American Society for Bone and Mineral Research Annual Meeting, September 12-15, 2014, Houston, Texas, USA. (Poster)
- **Ong A**, Weiler H, Wall M, Hamilton-Levitt ER, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. Dietary calcium versus calcium supplementation on vascular and bone health in postmenopausal women: results of a pilot study. Dietitians of Canada National Conference: Canadian Foundation of Dietetic Research, June 12, 2014, Ottawa, Ontario. (Oral)
- **Ong AM**, Weiler HA, Wall M, Haddad R, Hamilton-Leavitt ER, Daskalopoulou SS, Goltzman D, Morin SN. The effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women: results of a 12-month pilot clinical trial. McGill University, McGill Cardiovascular Research Day, May 5, 2014, Montreal, Quebec. (Poster)

- **Ong A**, Weiler H, Wall M, Haddad R, Daskalopoulou SS, Goltzman D, Morin SN. Dietary calcium versus calcium supplementation on vascular and bone health in postmenopausal women; preliminary results of a pilot study. Research Institute of the McGill University Health Centre, 10th Annual Retreat of the Musculoskeletal Axis, February 7, 2014, Montreal, Quebec. (Poster)

Contributions of Authors

For manuscript 1, the candidate was the primary author, wrote the first draft of the manuscript. The candidate was also involved in collection of the data and performed statistical analyses along with Michelle Wall and Rouba Haddad who were involved in the recruitment of participants. Jessica Gorgui contributed to the collection of vascular data and conducted laboratory work. Dr. Morin, Dr. Weiler, Dr. Goltzman and Dr. Daskalopoulou all made contributions to the design of the trial, statistical analyses and interpretation of the data as well as providing critical revisions to the manuscript. This research was funded by the Canadian Institutes of Health Research and study supplements were sponsored by Euro-Pharm International Canada Inc.

For manuscript 2, the candidate was the primary author, wrote the first draft of the manuscript and was involved in the design of the study (with Dr. Morin, Dr. Weiler, Dr. Goltzman, Dr. Daskalopoulou and Dr. Whiting), participant recruitment (with Michelle Wall), development of the calcium-focused food frequency questionnaire study (with Dr. Morin, Dr. Weiler, Dr. Goltzman and Dr. Whiting), data acquisition, analysis and interpretation of nutritional data. Dr. Morin, Dr. Weiler, Dr. Goltzman, Dr. Daskalopoulou and Dr. Whiting all made contributions to some aspect of statistical analyses or data interpretations as well as providing critical revisions to the manuscript. This research was funded by the Canadian Institutes of Health Research.

For manuscript 3, the candidate was the primary author and conducted the literature search and data synthesis (with Kai Kang), performed statistical analyses, made major data interpretation and wrote the first draft of the manuscript. The candidate, Dr. Morin and Dr.

Weiler were responsible for study concept and design. Dr. Morin and Dr. Weiler provided critical revisions to the manuscript.

For manuscript 4, the candidate was the primary author and wrote the first draft of the manuscript. The candidate led the intervention, and was also involved in recruitment of participants, collection of the data, laboratory work and performed statistical analyses. Dr. Morin, Dr. Weiler, Dr. Goltzman and Dr. Daskalopoulou all made contributions to the design of the trial, statistical analyses and interpretation of the data as well as providing critical revisions to the manuscript. This research was funded by the Canadian Institutes of Health Research and the Dairy Farmers of Canada.

Acknowledgments

There are many people that I would like to thank for their ongoing support that made this work possible. First and foremost, I would like to thank my co-supervisors, Dr. Suzanne Morin and Dr. Weiler, for their patience, guidance, encouragement, and mentorship. I have been grateful to be mentored by both of you, passionate and dedicated researchers, and allowed me to grow as a researcher during the process. I would also like to thank my supervisory committee members, Dr. David Goltzman and Dr. George Thanassoulis, for their expertise, insight, and constructive feedback during my training.

I would also like to thank all of the participants who have volunteered their time to participate in the studies presented in this thesis. Advances in science would not be possible without the dedication and contribution of participants.

Next, I would like to thank all the members of our laboratory group for their ongoing support and help with different aspects of the study. In particular, I would like to thank Michelle Wall not only for training me at the beginning of my degree, her tremendous help in the recruitment of participants or for managing the logistics of the studies, but also for being an amazing friend throughout the journey. I would also like to acknowledge Kristina Parsons, Priya Patel, and Sumra Kureishy for their assistance in participant recruitment, study visits, and data entry. I would also like to thank Caroline Joly and Nisha Patel for the daily encouragements and for providing a helping hand whenever it was needed.

I would also like to thank Sherry Agellon and Paula Lavery for their tremendous help with the laboratory work and for teaching me technical skills. I would also like to thank Dr. Daskaloupoulou, her staff and her students, Yessica Gomez-Sandoval, Alvin Kuate Defo, Shubhabrata Das, Huaien Zheng and Javiera Rematal, for their expertise and laboratory work.

My peers, May Slim, Anne-Julie Tessier, Véronique Ménard, Tamara Cohen, Nathalie Garibeh, Maryam Razaghi and Neil Brett, thank you all for your help, support and encouragements. One of the biggest rewards that I have gained from this journey is to have met each of you and to have developed an amazing friendship with you.

My best friends, Gigi, Winnie, Elaine, Barbara and Tom, thank you for helping me keep my head up high. This journey would have been very different and significantly more challenging without your encouragements, words of wisdom, and constant reminder that balance is the key to success in life.

I would also like to acknowledge my fur babies, Kali and Kumbha, who have kept me company throughout this entire journey, through good and bad times. Their unconditional love gave me the strength to move pass unforeseen challenges and to focus on my goals. They reminded me to cherish every moment and to be grateful every day. Another companion that I would like to acknowledge is my Lenovo Thinkpad T430s for being good with me over the last 6 years.

Last but not least, I would also like to thank my family for their patience and their understanding over the past few years, especially my beloved mother Connie.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Full text
24HR	24-hour recall
24HRs	4 nonconsecutive 24-hour recalls
25(OH)D	25-hydroxyvitamin D
BMD	Bone mineral density
BMI	Body mass index
BSAP	Bone-specific alkaline phosphatase
CAD	Coronary artery disease
CaDiet	Dietary calcium group
CaSuppl	Supplemental calcium group
CaMOS	Canadian Multicentre Osteoporosis study
cfPWV	Carotid-femoral pulse wave velocity
cIMT	Carotid intima-media thickness
crPWV	Carotid-radial pulse wave velocity
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
cfPWV	Carotid-radial pulse wave velocity
CONSORT	Consolidated Standards of Reporting Trials
CTX	C-terminal telopeptide of type 1 collagen
CVD	Cardiovascular disease
dCa_i	Dietary calcium intake

DRI	Dietary Reference Intake
DXA	Dual-energy x-ray absorptiometry
EAR	Estimated Average Requirement
ECG	Electrocardiogram
FFQ	Food frequency questionnaire
FFQ1	Food frequency questionnaire completed at baseline
FFQ2	Food frequency questionnaire completed at 1 month
FMP	Fermented milk product
GRADE	Grading of Recommendations, Assessments, Development, and Evaluation
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratio
HR-pQCT	High resolution peripheral quantitative computed tomography
hsCRP	High sensitivity C-reactive protein
HMW	High molecular weight
IL-6	Interleukin-6
IOM	Institute of Medicine
IQR	Interquartile range
L. reuteri 6475	*Lactobacillus reuteri* ATCCPTA 6475
LDL-C	Low-density lipoprotein cholesterol
NTX	N-terminal telopeptide of type 1 collagen
OPG	Osteoprotegerin
OR	Odds ratio
P1NP	Procollagen type 1 N-terminal propeptide

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTH	Parathyroid hormone
PWV	Pulse wave velocity
RANKL	Receptor activator of nuclear factor- κB ligand
RCT	Randomized controlled trial
RDA	Recommended Dietary Allowance
RR	Relative risk
SD	Standard deviation
SIDE	Software for Intake Distribution Estimation
TAG	Triacylglycerol
TRACP 5b	Tartrate-resistant acid phosphatase 5b
UL	Tolerable Upper Intake Level
WHI	Women's Health Initiative

CHAPTER 1

Introduction

1.1 Background and rationale

The pivotal role of calcium in bone mineralization is well established. As an essential nutrient, inadequate intakes are associated with lower bone mass and osteoporosis. In particular, during the menopause transition period, the need for calcium from the diet is increased to counter the accelerated bone loss. Approximately 2 million Canadians are affected by osteoporosis with a prevalence of 21.3% in women and causing 80% of all fractures in adults over the age of 50 years, predominantly postmenopausal women and the elderly (1, 2). Major sources of dietary calcium in the Canadian diet include milk, milk products, and fortified plant-based beverages. Other foods such as dark leafy greens and almonds also provide calcium, but relatively less per serving than dairy products. Despite the recognition of the important role of calcium in bone health, over 80% of Canadian women >50 years do not meet the Estimated Average Requirement (EAR, 1000 mg/day). The average calcium intake from food sources was ~800 mg/day in 2004 for women 51-70 years and ~700 mg/day for women ≥71 years (3).

Calcium supplements are economical and convenient alternatives to help meet the recommended intakes for calcium and national surveys suggest that over half of women in North America have taken calcium supplements (3, 4). Calcium supplements have shown a modest decrease in fracture risk and are accepted as an option for those who cannot meet their needs from dietary sources alone (5, 6). Calcium supplements are generally well tolerated but they can cause gastrointestinal discomfort and when intakes exceed the Tolerable Upper Intake Level (UL), there is an increased risk of renal lithiasis (7). However, results of two meta-analyses raised important concerns regarding the safety of calcium supplements with regards to cardiovascular health (8, 9). These reports suggested an increased risk of myocardial infarction in individuals who received calcium supplements without vitamin D (hazard ratio [HR] 1.31,

95% confidence interval [CI] 1.02-1.67) or with vitamin D supplementation (HR 1.26, 95% CI 1.07-1.47). Concerns have been raised over the methodology of these meta-analyses and other reports have documented opposite or inconclusive results (10-15). The available evidence was derived from studies that aimed at examining bone health endpoints as primary outcomes due to a lack of randomized controlled trials (RCTs) investigating the effect of supplemental calcium on cardiovascular outcomes. Clinical data from an ancillary study showed no difference in carotid intima-media thickness and carotid atherosclerosis between elderly women who were assigned to 1.2 g/day of supplemental calcium and those who took a placebo at 3 years (13). Interestingly, the authors found that participants in the highest tertile of total calcium intake had reduced carotid atherosclerosis compared with women in the lowest tertile (odds ratio [OR] 0.70, 95% CI 0.51-0.96). The evidence remains conflicting and the mechanism that underlies the speculated adverse effects of supplemental calcium on vascular health remains unknown.

On the other hand, dietary calcium intake is generally associated with favorable cardiovascular outcomes or no association (16-19). Previous studies have investigated the association between milk and milk product consumption with coronary heart disease (CHD) and a meta-analysis of 12 prospective cohort studies found no conclusive evidence to suggest an increased risk associated with dairy intake due to inconsistencies in the results from studies (19). Findings from other reports suggested that dietary calcium intake and milk product consumption are favorable to cardiovascular health (18, 20-24). Epidemiological studies have consistently reported an inverse association between dietary calcium intake and blood pressure (25-28), and no adverse effect on blood lipid profile has been documented (29-32). Prospective cohort studies of middle-aged and postmenopausal women have demonstrated an inverse relationship between the highest dietary calcium intake and incidence of ischemic heart disease (33, 34). Similarly,

evidence from observational studies have consistently reported an inverse association between milk product consumption and risk of stroke (20, 35, 36), cardiovascular disease (CVD) (37), and myocardial infarction (38).

However, recent findings from a large Swedish cohort study associated high dietary calcium intake and high milk intake with increased mortality (39, 40). The authors observed a dose-dependent higher mortality rate with milk intake, predominantly from cardiovascular death, whereas fermented milk product (FMP) consumption was associated with lower mortality risk. It was hypothesized that the higher content of D-galactose in milk may be pro-inflammatory, whereas the probiotic content found in FMP may exert anti-inflammatory properties to explain the protective association of FMP against mortality risk (40). Yet, results of a dose-response meta-analysis of prospective cohort studies showed no relationship between milk intake and mortality, CVD, or CHD (41). Differences in exposures and dietary collection methods as well as reverse causation may in part explain the inconsistencies observed in the Swedish cohort compared to other population groups.

Furthermore, the suggested pro-inflammatory effects of milk have not been observed in interventional studies. RCTs have not shown increases in circulating pro-inflammatory markers following interventions of milk or milk products, though the studies were conducted in selected populations of overweight subjects or adults with low-grade systemic inflammation (42, 43). Nonetheless, an inverse relationship between dairy product intake and C-reactive protein (CRP) concentrations has been reported in cross-sectional studies of healthy adults (22, 44). High dairy diets have been shown to increase adiponectin significantly, an anti-inflammatory adipokine, as compared to low-dairy diets in a blinded crossover study with obese subjects (45).

There is growing interest in the potential protective effects of FMP against chronic diseases, including osteoporosis and CVD. Although dairy foods are well recognized as calcium-rich foods, emerging research proposes that each type of dairy, such as milk, cheese and yogurt, exerts varying health effects based on their unique and complex matrices of nutrients and non-nutrients resulting from probiotics, fermentation and processing (46). Modest inverse associations of total FMP with all-cause mortality and CVD have been reported in a meta-analysis of 29 cohort studies (41). Findings of a recently published cohort study of Australian women also suggest an inverse association between FMP consumption and CVD risk (47).

In view of the uncertainty regarding the safety of calcium supplements on vascular health, dietary sources should be the preferred source of calcium. However, whether FMP provides more health benefits than non-FMP requires further investigation.

1.2 Statement of purpose

There is an urgent need for clinical trials to investigate the effect of supplemental calcium on vascular health related outcomes in postmenopausal women prospectively. The global aim of this thesis was to examine the differential effects of supplemental calcium versus dietary calcium on selected inflammatory markers and bone health biomarkers, as well as to investigate how different types of milk and milk products affect bone health and vascular health.

1.3 Objectives and hypotheses

Study 1: Feasibility study (12-month duration)

Primary objective: Determine the feasibility of controlled dietary and supplemental interventions of an RCT that aimed to assess the effect of supplemental calcium as compared with dietary calcium on vascular and bone health outcomes in healthy postmenopausal women.

Secondary objectives: Explore the differential effects between supplemental and dietary calcium on vascular and bone health biomarkers.

Hypothesis: It was hypothesized that high compliance to diet modifications and supplemental interventions would be achieved through monthly nutritional counseling, and the dietary intervention would associate with better outcomes such as reduced arterial stiffness, blood pressure, and blood lipid profile.

Study 2: Calcium-focused food frequency questionnaire validation study

Primary objective: Develop and evaluate the relative validity of a calcium-focused food frequency questionnaire (FFQ) to estimate usual dietary calcium and vitamin D intake of postmenopausal women in Montreal, designed for the purpose of estimating dietary calcium intake of postmenopausal women in a randomized controlled trial (Study 4).

Hypothesis: It was hypothesized that the semi-quantitative calcium-focused FFQ would be in agreement with the reference method in the assessment of dietary calcium intake.

Study 3: Systematic review and meta-analysis of fermented milk products on bone health

Primary objective: In a meta-analysis, investigate the effect of FMP on bone mineral density (BMD) and hip fracture risk in postmenopausal women or women ≥ 50 years.

Secondary objective: Explore the effect of FMPs on bone turnover markers.

Hypothesis: It was hypothesized that FMP consumption would associate with more benefits to bone health, such as greater BMD, lower incidence of fragility fractures, and lower levels of bone turnover markers.

Study 4: Randomized controlled trial (12-month duration)

Primary objective: Examine the effect of dietary and supplemental calcium intake on selected inflammatory and bone health biomarkers in healthy postmenopausal women.

Secondary objectives: Examine the effect of calcium, either from the diet alone or predominantly from supplements, at the Recommended Dietary Allowance (RDA) level, on body composition, and explore the association between different types of milk products with inflammatory and bone markers.

Hypothesis: It was hypothesized that the dietary calcium intervention would be associated with a more favourable effect on markers of inflammation and bone health markers than a calcium intake predominantly derived from supplements.

CHAPTER 2

Literature Review

2.1 Introduction

2.1.1 Biological role and metabolism of calcium in humans

Calcium is an abundant mineral in the human body accounting for 2-4% of body weight and 99% of the calcium in the body is stored in bone tissue and teeth as hydroxyapatite (48, 49). The remaining calcium is found in the circulatory system, extracellular fluid, and soft tissues to mediate biological processes as a secondary messenger such as muscle contraction, vasodilation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion (50).

Although calcium intake from food sources and nutritional supplements is a major determinant of calcium balance, calcium ion concentration in the extracellular fluid is tightly regulated by effector loops involving the parathyroid hormone (PTH), calcitonin and 1,25-dihydroxyvitamin D (49). PTH is the primary and acute endocrine hormone responsible for calcium homeostasis. When there is a fall in calcium concentration, PTH is released from the parathyroid glands to restore ionized calcium levels by stimulating bone resorption. The release of PTH also acts on the kidney to increase renal reabsorption of filtered calcium and to increase the activity of vitamin D 1α-hydroxylase thereby increasing the synthesis of 1,25-dihydroxyvitamin D, also known as calcitriol (51). A key function of calcitriol is to regulate calcium homeostasis through actions in the intestine, kidney and bone. Calcitriol increases blood calcium levels by increasing dietary calcium absorption from the gastrointestinal tract and renal tubular reabsorption of calcium, and by promoting the activation of osteoclasts to release calcium from bone (52). In contrast, when serum calcium levels rise above normal, the thyroid gland releases calcitonin to inhibit osteoclastic activity, increase calcium excretion and inhibit 1α-hydroxylase activity. In summary, calcium homeostasis is a hormonally regulated integrated process that mediates bone turnover, renal calcium excretion and intestinal absorption (53).

2.1.2 Calcium and bone integrity

The skeleton plays a structural role and is also the body's mineral supply. Bone remodelling is the natural process of bone resorption to break down old bone followed by formation to replace with new bone. Bone balance changes throughout the normal lifespan, depending on the relative rates of bone formation and resorption. Under normal conditions and adequate nutrition, bone formation is greater than bone resorption from birth to late adolescence to allow skeletal growth (54). Once peak bone mass is achieved in young adult years, bone turnover is neutral with a balance between osteoblastic and osteoclastic activity. The non-structural role of calcium stored in the bones during this period is to offset obligatory losses of calcium through sweat, desquamated skin, and excreta (49). Later in life, the effects of the aging process leads to a gradual and progressive decline of bone mineral density (BMD) as a result of decreased bone formation, increased bone resorption, secondary hyperparathyroidism, and reductions in both intestinal and renal tubular absorption of calcium (55). Although age-related bone loss occurs in both men and women, women experience an accelerated loss of BMD at menopause and may persist up to 10 years after menopause (55). The effect of estrogen deficiency on bone is a loss of control over mediators of bone resorption, such as inhibition of osteoclastic activity and suppression of the production of bone resorbing cytokines (55, 56). Osteoporosis, a systemic skeletal disease, occurs when there is a decrease in either the quantity or quality of bone, or both, leading to increased bone fragility and an increased susceptibility to fractures. In Canada, the prevalence of osteoporosis is estimated to be 21.3% in women and 5.5% in men over the age of 50 y (1). A major clinical consequence of osteoporosis is the increased risk of fragility fractures, which are becoming increasingly common in older women and men, resulting in increased morbidity and mortality (57). The pathogenesis of osteoporosis is

multifactorial and risk factors include increasing age, excess alcohol consumption, smoking, genetics, sedentary lifestyle, poor diet quality and inadequate intakes of calcium and vitamin D. As a result, calcium and vitamin D supplementation has been widely recommended to prevent osteoporosis and fragility fractures.

2.1.3 Calcium requirements

Calcium is one of 21 essential nutrients required by humans in relatively larger quantities in the diet compared to other nutrients. Given that the body can only store a finite amount of calcium, a continuous adequate calcium intake is crucial to meet the needs to support the structural role of the skeleton and to provide the quantities needed for cellular functions. Using the body of evidence surrounding bone health outcomes, the National Academy of Medicine (formerly called the Institute of Medicine) developed new Dietary Reference Intakes (DRIs) for calcium (**Table 2.1**). The Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) for 1 year through 50 years of age relied primarily on calcium balance studies, whereas the EARs and RDAs for postmenopausal women are higher to reflect the greater loss of bone mass due to the effect of menopause (58). The Tolerable Upper Intake Level (UL) for adults was based on data related to the incidence of renal lithiasis which were mainly derived from research with postmenopausal women who took calcium supplements.

Table 2.1 Dietary Reference Intakes (DRIs) for calcium (mg/day) by life stages

Life Stage Group	EAR	RDA	UL
Infants			
0-6 mo	200*	200*	1000
6-12 mo	260*	260*	1500
Children			
1-3 y	500	700	2500
4-8 y	800	1000	2500
Males			
9-13 y	1100	1300	3000
14-18 y	1100	1300	3000
19-30 y	800	1000	2500
31-50 y	800	1000	2500
51-70 y	800	1000	2000
>70 y	1000	1200	2000
Females			
9-13 y	1100	1300	3000
14-18 y	1100	1300	3000
19-30 y	800	1000	2500
31-50 y	800	1000	2500
51-70 y	1000	1200	2000
>70 y	1000	1200	2000

EAR, Estimated Average Requirement; RDA, Recommended Dietary Allowance; UL, Tolerable Upper Intake Level

* Adequate Intakes (AI) are recommended when there is not sufficient evidence available to establish an EAR. The AI covers the needs of all healthy individuals in the group (50).

2.2 Calcium Sources

2.2.1 Dietary calcium

In Canada, milk and milk products are the main contributors of calcium in the diet (**Table 2.2**). Aligning with the DRIs set by the National Academy of Medicine, *Milk and Alternatives* was a standalone food group in the 2007 Canada's Food Guide (59). One serving of milk (250 ml), yogurt (175 g), or cheese (50 g) generally provides 300 mg of calcium. Greek yogurts, a style of yogurt that has emerged into the marketplace in recent years, have a calcium content that can range from 100 mg to 500 mg per portion, depending on the manufacturers. Greek yogurts are different from regular yogurts not only from their sensory properties, but the former type generally has a higher protein content per serving than regular yogurts.

Beyond calcium, individual dairy products are unique in their nutrient and non-nutrient compositions as a result of fermentation and processing. Dairy products can be further categorized as fermented milk products (FMPs), which include cheese and yogurts, and non-FMPs. There is growing interest in research to demonstrate the varying health effects of FMPs, such as protective effects against cardiovascular disease (CVD), diabetes, and fractures (46). These advances in science suggest that future dietary guidelines may need to consider each dairy type separately based on their different effects on health.

Calcium is also found in many non-dairy sources, either naturally or from fortification. In Canada, mandatory fortification of foods with calcium for adults is limited to meal replacement products and nutritional supplements (60). Under the Food and Drugs Act of Health Canada, voluntary fortification with calcium is permitted for some foods, including flour, plant-based beverages, and orange juice (60, 61). Plant-based beverages fortified with calcium serve as milk alternatives and also provide approximately 300 mg of calcium per serving of 250 ml (62). Non-

enriched soy beverages also contain calcium, but only provides 80 mg per 250 ml (62). Tofu prepared with calcium provides 100-300 mg of calcium per serving whereas soft tofu only provides 80 mg per serving (62). Canned salmon with bones contain approximately 200 mg calcium per 75 g serving (62).

Table 2.2 Calcium content of some common foods in Canada[1]

Food	CFG Serving size[2]	Calcium (mg)	
		Per CFG serving[2]	Per 100 g serving
Milk and milk products			
Fluid milk	250 mL	321	124
Plain yogurt	175 g	294	168
Fruit yogurt	175 g	228	130
Hard cheese	50 g	535	1070
Firm cheese	50 g	343	687
Soft cheese	50 g	148	297
Non-dairy foods			
Plant-based beverage fortified with calcium	250 mL	333	129
Tofu prepared with calcium	150 g	290	264
Canned salmon with bones	75 g	191	255
Kale, raw	250 mL	96	135
Spinach, raw	250 mL	31	99
Almonds	60 mL	76	253
Broccoli	125 mL	44	47
Beans, cooked	175 mL	55	47
Orange	1 medium fruit	65	43
Bread	1 slice (35 g)	45	129

CFG, Canada's Food Guide 2007.
[1] Values presented are averages calculated items included in the Canadian Nutrient File 2015 (59, 62).
[2] Servings sizes as per the 2007 Canada's Food Guide (63).

2.2.2 Calcium supplements

There are different forms of calcium supplements and they vary considerably in characteristics and costs. The most common forms are calcium carbonate and calcium citrate, and they have comparable bioavailability (34.2% vs. 37.9% absorption efficiency per 300 mg load, respectively) (64). Calcium carbonate is associated with more gastrointestinal side effects, whereas calcium citrate is generally better tolerated (65). For that reason, it is recommended to take calcium carbonate with meals.

2.2.3 Trends in calcium intake in postmenopausal women

Data from the 2004 Canadian Community Health Survey (CCHS) showed that over 80% of Canadian women >50 years had a calcium intake below the EAR from dietary sources (3). More specifically, the average dietary calcium intake was 751 mg/day (95% CI 725-776) for women of 50-70 years and 689 mg/day (95% CI 659-719) in women >70 years. Among users of supplements containing calcium (49% and 45.9% of women of 50-70 years and >70 years, respectively), the prevalence of total calcium intake below the EAR ranged from 26.9% to 33.3%. Milk and milk products were the main dietary sources of calcium, consistent with national nutritional recommendations which have been advocating daily consumption of milk and milk alternatives to effectively obtain calcium and vitamin D (59). However, comparison of dietary data of the 2015 survey cycle of the CCHS showed no improvement in the overall consumption of milk and milk alternatives in 2015 compared to 2004 in older adults ≥55 years with a national average of 1.4 servings/day in both survey cycles (66). Although the report did not explicitly examine the adequacy of calcium intake, these observations may imply low intake of calcium since milk and milk products such as yogurt and cheese are also major contributors of dietary calcium in Canada. Thus, these findings suggest that inadequate calcium intake remains a public

health issue among Canadians, especially in older women (66, 67).

Use of calcium supplements have declined by 5.5% in 2015 from 2004 among women >50 years (68). The combination of low dietary calcium intake and decreased use of calcium supplements puts more women at risk of calcium inadequacy, and consequently has the potential to increase the prevalence of osteoporosis and incidence of fragility fractures. The decrease in calcium supplement use may likely have been the result of the uncertainty regarding the safety of supplemental calcium on cardiovascular risk, which became a public concern following the publication of a meta-analysis in 2010 and will be discussed in the next section.

2.3 Association of calcium intake with vascular health risk

2.3.1 Dietary calcium studies and vascular health outcomes

CVD is the second leading cause of mortality in women >50 years globally, with coronary artery disease (CAD) and stroke representing 23% and 18% of cause of death, respectively (69, 70). Most epidemiological studies have demonstrated an inverse association between dietary calcium intake and risk factors of CVD, including blood pressure (25-28) and blood lipid profile (29-32). Similarly, studies have shown an inverse association between consumption of dairy foods, such as milk, yogurt, and cheese, and vascular health markers and related outcomes. Data from observational studies have shown that higher consumption of dairy associated with more favorable measurements of blood pressure (71-73), arterial stiffness (74-76), and carotid intima-media thickness (cIMT) (77) compared to lower intakes. Some reports suggested that dairy food consumption may also be inversely associated with the risk of stroke (20, 35, 36), CVD risk (37), and myocardial infarction (78) . The evidence thus far suggests that dietary approaches to increasing calcium intake are favorable to cardiovascular health (18, 20-23, 79).

However, the relation between dietary calcium intake and dairy food intake and CVD and related mortality is supported by contradictory or inconclusive evidence in women. Findings from a large prospective cohort study (*n*=61,433), with an average follow-up of 19 years, suggested a trend in increased mortality from all-causes (HR 1.40, 95% CI 1.17-1.67) in Swedish women who consumed ≥1400 mg/day of dietary calcium (39), and total calcium intake from dietary and supplemental sources ≥1400 mg/day was associated with all-cause (HR 1.40, 95% CI 1.25-1.57) and cardiovascular mortality (HR 1.51, 95% CI 1.23-1.84). Evidence from other prospective cohort studies and meta-analyses did not support the suggested risk of mortality associated with high dietary calcium intake, though high intakes were less common in other cohorts (15, 80-83). In the Canadian Multi-center Osteoporosis Study (CaMOS), a population-based longitudinal cohort (*n*=9423) with a 10-year follow-up, high calcium intake (>1200 mg/day) did not associate with all-cause mortality (80). On the contrary, the results suggested a beneficial trend towards reduced mortality risk (HR 0.95, 95% CI 0.89-1.01) per 500 mg increase in daily calcium intake, up to 1000 mg/day, among women. Nonetheless, low calcium intake associated with increased risk of mortality in both Swedish and Canadian cohort studies. The UK Biobank cohort (*n*=497,828) also examined the association of dietary calcium intake with mortality over an average follow-up of 4.2 years (15). Dietary calcium intake did not associate with all-cause or cause-specific mortality (*P*=0.80). However, dietary calcium intake data was derived from a single 24-hour recall in a sub-sample of participants (*n*=68,795) whereas a FFQ was used to estimate usual dietary calcium intake in the Swedish Mammography Cohort and the CaMOS cohort. The inconsistency in the evidence may be due to the scarcity of data regarding very high total or dietary calcium intakes, differences in dietary assessment methods and duration of follow-up, or possibly influences by reverse causation.

The relation between milk intake with mortality risk was also examined in the literature. Results from two large Swedish cohorts suggested that milk intake is associated with higher mortality in women and men. For every glass of milk, the adjusted HR of all-cause mortality was 1.15 (95% CI 1.13-1.17) in women and 1.03 (95% CI 1.01-1.04) in men (84). Interestingly, consumption of FMPs such as yogurt and sour milk inversely associated with mortality in women. A hypothesized explanation for the varying effects between non-FMPs and FMPs on CVD and mortality may be related to the higher lactose and galactose content found in milk, which is believed to induce oxidative stress and inflammation (84). A dose-dependent higher mortality rate with higher milk intake was observed, predominantly from cardiovascular death, and positive associations between milk intake and markers of inflammation over a 20-year period were documented (40).

Multiple reports have since been published and found opposite or null effect of milk on cardiovascular risk (83, 85, 86). For instance, self-reported milk consumption was inversely associated with all-cause mortality (OR 0.92, 95% CI 0.86-0.98) but not with cardiovascular mortality (0.93, 95% CI 0.81-1.07) among participants of the UK Biobank cohort. However, the inverse association between milk consumption and all-cause mortality was only borderline in younger participants (OR 0.93, 95% CI 0.86-1.00) and null in older participants (OR 0.99, 95% CI 0.89-1.11) following stratification by age group (<65 years and ≥65 years) (86). In a large multinational prospective cohort study with a median follow-up of 9.1 years, higher milk intake (>1 serving/day) associated with a reduced risk of composite of death or CVD (HR 0.90, 95% CI 0.82-0.99) (83). On the other hand, there were some consistent evidence to support the differential health effects of FMPs and non-FMPs. For instance, consumption of FMPs such as cheese, yogurt and fermented milk was associated with modestly lower risk of total mortality and

CVD, with 2% lower risk of each per serving of 20 g/day (41). Another report found no consistent association between non-FMP or FMP consumption on mortality (87).

The overall evidence indicates a suggestive beneficial effect of dairy food consumption, especially from FMPs, on cardiovascular health. The inconsistent findings may be explained by the overall higher dairy consumption in the Swedish cohort, Sweden being the third highest consumers in the world (88). The benefits exclusively associated with FMP are an emerging area of research, yet research on the impact of dairy product consumption has often explored the effect of total dairy intake. Thus, there is an urgent need for future studies to examine specific dairy product types separately to better understand their individual impact on health.

2.3.2 Calcium supplementation studies and cardiovascular health outcomes

Findings from meta-analyses of randomized controlled trials (RCTs) have suggested small but significant improvements in vascular risk factors, such as blood pressure (89-92) and serum lipid concentrations (93), following calcium supplement interventions. Yet, the effect of calcium supplementation, with or without vitamin D co-supplementation, on blood pressure reduction could not be confirmed by other meta-analyses of RCTs (94-96) and the mechanisms have not been clearly established. On the other hand, potential adverse effects of calcium supplementation on vascular calcification and mortality have been documented in patients with renal compromise (97). Reports have suggested that supplemental calcium may also be associated with increased cardiovascular health risk in generally healthy women with no renal impairment, but uncertainties remain due to inconsistent findings and the lack of high quality evidence to prospectively examine the effect of calcium supplementation on the vasculature. Results of an RCT and two subsequent meta-analyses of randomized trials reported increased risk of cardiovascular events associated with calcium supplementation in women (8, 9, 98). The

retrospective meta-analyses of RCTs indicated an increased risk of myocardial infarction in individuals who received calcium supplements without vitamin D (HR 1.31, 95% CI 1.02-1.67) (8) or with vitamin D (HR 1.26, 95% CI 1.07-1.47) (9). However, other reports have documented a lack of effect as shown in **Table 2.3** (14, 81, 99, 100).

The inconsistent evidence may be explained by multiple reasons. Methodological differences were especially flawed in the two meta-analyses that had initially raised the concern regarding the safety of calcium supplements. Furthermore, studies included in these meta-analyses were limited by inadequate compliance with calcium supplement intervention, use of non-trial calcium supplements, potential bias in event ascertainment, and lack of adjustment for known CVD determinants. Furthermore, the RCTs were not originally designed to examine cardiovascular endpoints as their primary or secondary outcomes. Instead, most studies were conducted in the context of osteoporosis prevention. The dosage and adherence to the study supplements varied across studies. A major limitation of calcium supplementation studies is the rare consideration for the participants' usual dietary calcium intake. Consequently, total calcium intakes, which is the sum of calcium from study supplements and background calcium intake, often exceed the RDA and often approach or even exceed the UL. The latter case may require more careful consideration since a total calcium intake below the UL was not shown to be associated with CVD risk in generally healthy adults (81).

If supplemental calcium is adversely associated with vascular health, the mechanism underlying these effects remains unclear. One suggested mechanism is via a progressive hypercalcemia-mediated arterial calcification and aortic stiffness (101, 102). An ancillary study of an RCT assessed the effect of 1.2 g/day of supplemental calcium on cIMT and carotid atherosclerosis but found no increase in either subclinical or clinical atherosclerosis compared to

placebo in elderly women after 3 years (13). There remains important knowledge gaps urging the need for prospective calcium trials to provide high level evidence elucidating the effect of supplemental calcium on vascular health in postmenopausal women.

Table 2.3 Results of meta-analyses examining the effect of supplemental calcium on cardiovascular risk

Authors and year	Number of studies	Dosage of calcium	Trial duration	Meta-analysis results (RR or OR, 95% CI)	Direction of findings (risk) and overall quality or risk of bias of trials[1,2]
Bolland et al. 2010 (8)	15 RCTs	0.5-2 g/day	2-5 y	<u>*Calcium only*</u> MI: RR 1.27 (1.01-1.59) Stroke: RR 1.12 (0.92-1.36) MI, stroke, or sudden death: RR 1.12 (0.97-1.30)	↑ MI, ↔ Stroke, ↔ MI, stroke or sudden death The authors provided a narrative description of the quality of the studies. All studies were double blinded, randomized trials. Few studies may be at higher risk of selection bias and other biases. Calcium with vitamin D co-supplementation trials were not included.
Bolland et al. 2011 (9)	9 RCTs	0.5-1.5 g/day	2-5 y	<u>*Calcium with or without vitamin D co-supplementation*</u> MI: RR 1.24 (1.07-1.45) Stroke: RR 1.15 (1.00-1.32) MI or stroke: RR 1.15 (1.03-1.27)	↑ MI, ↔ Stroke, ↑ MI or stroke The authors did not assess the quality of the included RCTs, but 8 of the nine studies were included in their previous meta-analysis (8), with the inclusion of studies with vitamin D co-supplementation. However, 46% of participants from the Women's Health Initiative Calcium/Vitamin D Supplementation Study were excluded from the analysis.
Mao et al. 2013 (99)	9 RCTs	0.6-1.2 g/day	1-7 y	<u>*Calcium only*</u> Major CV events: OR 1.16 (0.97-1.40) MI: OR 1.28 (0.97-1.68) Stroke: OR 1.14 (0.90-1.46) <u>*Calcium with vitamin D co-supplementation*</u> Major CV events: OR 1.06 (0.94-1.19) MI: OR 1.06 (0.92-1.21) Stroke: OR 0.98 (0.86-1.13)	↔ Major CV events, ↔ MI, ↔ Stroke Quality of studies based on Jadad scores: 4 studies were of low quality and 7 studies were of high quality. The median score was 4 (range: 1-4).
Lewis et al. 2015 (14)	18 RCTs	0.5-1.6 g/day	1-7 y	<u>*Calcium with or without vitamin D co-supplementation*</u> CHD events: RR 1.02 (0.96-1.09) CHD mortality: RR 1.04 (0.88-1.21) MI: RR 1.08 (0.92-1.26) Angina pectoris: RR 1.09 (0.95-1.24) Chronic CHD: RR 0.92 (0.73-1.15)	↔ CHD events or mortality, ↔ MI, ↔ Angina pectoris, ↔ Chronic CHD Risk of bias of studies: 6 trials were at higher risk of performance and detection bias, 4 of which were also at a higher risk selection bias.

Table 2.3 (Continued)

Authors and year	Number of studies	Dosage of calcium	Trial duration	Meta-analysis results (RR or OR, 95% CI)	Direction of findings (risk) and overall quality or risk of bias of trials[1,2]
Jenkins et al. 2018 (103)	20 RCTs	Not reported	6 mo to 8 y	*Calcium only* All-cause mortality: RR 1.08 (0.97-1.21) CVD mortality: RR 1.24 (0.27-5.65) Total CVD risk: RR 1.43 (0.79-2.59) MI risk: RR 1.69 (0.94-3.04) *Calcium and Vitamin D* All-cause mortality: RR 0.95 (0.90-1.01) CVD mortality: RR 0.92 (0.77-1.11) Total CVD risk: RR 1.03 (0.94-1.12)	↔ All-cause mortality, ↔ CVD mortality, ↔ Total CVD risk, ↔ MI risk The authors used GRADE to evaluate the quality of the evidence for each outcome. *Calcium only* Very low quality for CVD mortality and total CVD risk. Low quality for MI Moderate quality for all-cause mortality *Calcium and Vitamin D* Low quality for CVD mortality and total CVD risk Moderate quality for all-cause mortality
Yang et al. 2019 (104)	16 RCTs	1-1.2 g/day	6 mo to 7 y	*Calcium with or without vitamin D co-supplementation* CVD risk: RR 0.99 (0.93-1.05) CHD: RR 1.08 (1.02-1.22) Stroke: RR 0.85 (0.61-1.20) MI: RR 1.14 (1.05-1.25)	↔ Stroke, ↔ CVD, ↑ MI, ↑ CHD Quality of studies based on Jadad scores: 3 studies were of low quality and 13 studies were of high quality. The median score was 4 (range: 1-5).

CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; OR, odds ratio; RCT, randomized controlled trials; RR, relative risk

[1] Methodological quality of trials were assessed by the authors of the respective systematic reviews and meta-analyses. When such data is not available, a summary statement of the overall quality is provided.

[2] Jadad score is based on randomization, concealment of treatment allocation, blinding, completeness of follow-up, and the use of intention-to-treat analysis. Studies with a score between 0 and 2 were considered to have low quality and studies with a score between 3 and 5 were considered of high quality (105, 106).

[3] Quality and risk of bias assessed using the Risk of Bias Tool developed by the Cochrane collaboration. Domains included random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data addressed, and selective reporting (107).

2.4 Inflammation, cardiovascular health and bone health

2.4.1 Inflammation and cardiovascular health

Along with vascular calcification, endothelial dysfunction and intimal thickening, inflammation is one of the main features of atherosclerosis (108-110). Pre-clinical models have demonstrated a spatial and temporal relationship between inflammation and vascular calcification, in which inflammation dominates in the early stages of atherosclerosis while osteogenic activity predominates in the later phase in arterial calcification (111). Micro-calcification, the initial deposition of calcium hydroxyapatite, may induce further inflammatory responses (112).

Several inflammatory markers have been associated with coronary arterial calcification, CVD and mortality, including CRP, cytokines and adipokines (113-116). Elevated levels of inflammatory markers, particularly CRP, the most extensively studied marker of inflammation, indicate an increased risk of CHD (117). CRP is one of the most sensitive acute phase reactants that is produced predominantly by the liver in response to high concentrations of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (118). Although mounting evidence suggests that CRP may not play a direct pathogenic role in atherogenesis, elevated concentrations of CRP have been consistently shown to be a prognostic marker of adverse cardiovascular events independent of the ratio of total cholesterol to high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, or other risk factors (119-122). The possible mechanistic role of CRP in atherogenesis is highly complex, exerting pro-atherogenic effects in many cells involved in plaque deposition (123). For instance, CRP may facilitate the adherence and migration of monocytes into vessel wall (124). CRP also catalyzes the pro-inflammatory trigger in plaque deposition by M1 macrophage polarization contributing to plaque infiltration

and in atherosclerotic lesions (125). Prospective epidemiological studies have demonstrated a consistent relationship between elevated serum and plasma concentrations of CRP and prevalence of subclinical atherosclerosis, risk of recurrent cardiovascular events, and incident cardiovascular events (126-132). Similarly, epidemiological evidence supports the value of high sensitivity CRP (hsCRP) in predicting CVD and vascular disease progression (116, 126). The addition of hsCRP concentration to the global risk assessment by the Framingham Risk Score has been shown to improve the prediction of CVD events (131, 133, 134).

Although there is extensive evidence indicating CRP as an important inflammatory biomarker associated with increased CAD, IL-6 plays an earlier and more central role in pro-inflammatory regulation process than CRP (135). Elevated concentration of IL-6 is one of the most potent drivers of CRP production and is released from vascular smooth muscle cells in response to atherosclerosis (136) Evidence from the literature has shown a positive association between high circulating concentrations of IL-6 and severe carotid stenosis, myocardial infarction, stroke, mortality and progression to heart failure (137-144). Furthermore, two large meta-analyses incorporating genetic and biomarker data have confirmed the crucial role played by IL-6 and IL-6 receptors (IL-6R) in the generation of inflammation and a dose-dependent association with CHD risk (145, 146). Several clinical trials have demonstrated the effectiveness of anti-inflammatory therapeutic agents that target IL-6 and IL-6R in decreasing the risk of cardiovascular-related events (147, 148).

Accumulating evidence indicates that adipokines, which are biologically active proteins secreted by adipose tissues, also exert pro-inflammatory and anti-inflammatory effects (149, 150). Leptin is an adipokine that plays a critical role in the regulation of energy balance and its concentrations are directly associated with adipose tissue mass (151). Unbalanced secretion of

leptin may contribute to impaired insulin signaling and a state of inflammation leading to the development of metabolic syndrome. For instance, leptin-induced pro-inflammatory and atherogenic effects include vascular inflammation, thrombosis, arterial stiffness, vascular smooth muscle hypertrophy, angiogenesis and hypertension (152, 153). Leptin concentrations have been associated with arterial stiffness and higher leptin concentrations have been found in patients with uncontrolled hypertension compared to those with controlled blood pressure (154, 155). Moreover, higher plasma concentrations of leptin were found to be inversely associated with vasodilation in resistance arteries in the elderly population (156), suggestive of a mediating role of leptin in the regulation of endothelial function contributing to the development of aortic mechanical dysfunction and arterial stiffness. Several possible mechanisms have been proposed, such as an enhanced neointimal and medial thickening in injured arterial walls, altered modulation of the renin-angiotensin-aldosterone system, stimulation of the proliferation and migration of vascular smooth muscle cells, and abnormal generation of reactive oxygen species (157-160). Observational studies have found positive associations of leptin levels with the severity and lesion complexity of coronary atherosclerosis, as well as poor clinical outcomes related to ischemic and hemorrhagic strokes (161). Serum leptin concentrations may strongly predict a first-ever acute myocardial infarction, and correlates with the number of diseased vessels in patients with acute myocardial infarction (162, 163). However, a meta-analysis of case-control and nested-case control studies reporting a lack of association between leptin levels and risk for CHD and stroke (164). It is possible that the association of leptin with CHD is largely dependent on BMI (165), but studies with obese and non-obese adults have shown leptin levels to be an independent predictor of cIMT and carotid plaque instability (166, 167). Although the detrimental effects of leptin on the cardiovascular system requires more thorough

investigation, the overall body of evidence indicates that hyperleptinemia associates with the presence and severity of CHD, stroke, increased cIMT and carotid plaque instability (152).

In contrast to leptin, plasma adiponectin is inversely related to visceral fat mass and BMI (168). Experimental evidence has shown that adiponectin has anti-atherogenic and anti-inflammatory properties, with demonstrated protective effects against endothelial dysfunction, atherosclerosis and hypertension (169, 170). Adiponectin circulates at high concentrations in different isoforms (high, middle, and low molecular weight) in healthy individuals (171). Increased levels of adiponectin are generally associated with decreased risk for CVD (151, 172). Similarly, adiponectin levels are significantly lower in patients with CAD compared with healthy subjects (173), albeit previous meta-analyses reporting conflicting results with regards to the association between adiponectin and CHD (174, 175). The conflicting results may be due to the heterogeneity in the assessment of different isoforms of adiponectin given that the high molecular weight isoform is the most physiologically active (171). Findings of a meta-analysis of observational studies suggest that adiponectin provides cardio-protective benefits in the early development of atherosclerosis, but only in selected populations (176). In patients with severe carotid stenosis, adiponectin associated with lower risk of adverse cardiovascular events and inversely associated with chronic low-grade inflammation (177). Adiponectin may be considered as a biomarker of metabolic compensation where hyperadiponectinaemia may be a signal of an anti-inflammatory response to CVD severity (150, 177).

Although the evidence is mainly derived from observational studies with some inconsistent results, there is strong evidence to link CRP, IL-6 and leptin with worse cardiovascular-related clinical outcomes, and adiponectin as a cardio-protective mediator.

2.4.2 Inflammation and osteoporosis

The role of inflammatory markers on bone remodeling have been observed in *in vitro* and in animal studies (178). The effects of inflammation on the pathogenesis of osteoporosis result in stimulation of osteoclastic activity and inhibition of osteoblastic activity (179). There is also considerable evidence from prospective observational studies identifying a relationship between elevated levels of inflammatory biomarkers with bone loss and risk of non-traumatic fractures in older adults (180-184), though the relationship may vary between inflammatory markers. Data on the longitudinal relationship between inflammatory markers and BMD in particular are lacking.

Cross-sectional studies found no association between IL-6 and BMD in postmenopausal women (185, 186). Yet, prospective studies have consistently shown IL-6 to be positively associated with greater bone loss (180, 187) and increased risk of hip fractures (183, 188). Although few studies reported an association between CRP and low BMD in older women (180, 189), the literature consistently showed an inverse relationship between CRP and incidence of non-traumatic fractures in elderly women (184, 190-194).

Leptin is involved in bone metabolism in various ways through both central, neureoendocrine signaling pathway and peripheral pathways (195). Via the central pathway, leptin inhibits bone formation via the hypothalamus and sympathetic nervous system by either suppressing osteoblast proliferation or by promoting osteoclastic activity via increasing the expression of the receptor activator nuclear factor-κB ligand (RANKL) (196-199). In contrast to the antiosteogenic effects of leptin via the central pathway, leptin secreted into the peripheral circulation by adipose tissue has anabolic actions on bone metabolism through interaction with the bone marrow mesenchymal stem cells, osteoblasts, osteoclasts and chondrocytes (200-202).

However, the exact role that leptin plays in the development of osteoporosis remains unclear. Further investigation is required to elucidate the complex mechanism underlying the regulations of leptin on bone metabolism. Previous investigations in obese, leptin-deficient mice suggest that leptin signaling on bone differs among different skeletal regions as well as between cortical and trabecular bone (203). Epidemiological evidence on the association between leptin concentrations and BMD are mixed. In a study in postmenopausal women without central obesity, leptin concentrations negatively associated with total hip BMD and femoral neck BMD (204). However, other observational studies reported either no association (205, 206) or a positive association with BMD in postmenopausal women (207-209). The discordant findings on the association of leptin concentrations with BMD may be confounded by body composition parameters such as body mass index (BMI) and fat mass (210). Currently, there is limited data examining the relation between circulating concentrations of leptin and non-traumatic fracture risk. Longitudinal evidence from one cohort study showed a reduced risk of nontraumatic fracture in the middle (RR 0.26, 95% CI 0.09-0.70) and highest tertile (RR 0.25, 95% CI 0.09-0.74) leptin groups compared to the lowest tertile group after adjustment for body weight and other potential confounding variables (P=0.02), suggesting that higher levels of serum leptin may be associated with lower nontraumatic fracture risk independent of body weight (211).

Adiponectin also influences bone metabolism by inducing osteoclast proliferation and differentiation (212). Adiponectin increases osteoclast activity via the activation of RANKL and inhibition of osteoprotegerin (OPG), resulting in reduction in BMD (212). Evidence from a meta-analysis showed that adiponectin consistently negatively associates with BMD of the lumbar spine, total hip, and total body in postmenopausal women and men, independent of fat mass and BMI (210). Similarly, one study observed a greater annualized rate of hip BMD loss of

−0.67% in the highest tertile of serum total adiponectin (range: 16.0-49.0 μg/mL) compared to −0.43% in the lowest tertile (range: 1.0-9.0 μg/mL) in elderly women (*P*-trend=0.019) (206). Observational data showed that higher levels of adiponectin (range: 11.0-53.0 μg/mL) may be predictive of high risk of fractures compared with lower levels (range: 1.0-6.0 μg/mL) in men (*P*-trend=0.007), but the relationship between adiponectin levels and fracture risk in postmenopausal women is supported by mixed data from cross-sectional observations (213, 214). Furthermore, little is known regarding the actions of the adiponectin isoforms in bone metabolism. The few studies that have evaluated the association between high molecular weight (HMW) adiponectin and bone health demonstrated that BMD was significantly associated with total adiponectin rather than HMW adiponectin (215-217). HMW adiponectin also negatively associated with BMD of the lumbar, femoral neck in postmenopausal women without central obesity (204), yet no effect on bone mass was observed in other studies (218).

Despite the lack of clinical studies examining a causal relationship, the current body of evidence is consistent and suggests that reducing systemic inflammation may represent an important approach to reduce fracture risk in population groups at high risk of osteoporotic fractures.

2.4.3 Link between osteoporosis and cardiovascular disease

Osteoporosis and CVD are two major public health problems, both associated with high morbidity and mortality. Many studies have shown an association between CVD and osteoporosis, suggesting that low BMD may be associated with CVD, subclinical measures of atherosclerosis, cardiovascular events and mortality (219-221). Similarly, a recent meta-analysis of cross-sectional and prospective studies reported that subjects with prevalent subclinical CVD had higher risk for increased bone loss and fractures than subjects without CVD (222),

suggesting a possible bi-directionality between the bone and vascular systems (220). Epidemiological data from cross-sectional and prospective cohort studies have consistently shown an inverse association between BMD and aortic calcification (223-231). Similarly, prospective cohort studies reported that severe aortic calcification associates with fragility fractures (230, 232-236). Severe aortic calcification may be associated with a 2.3-fold increase risk in proximal femur fractures (OR 2.3, 95% CI 1.1-4.9) (230), 3.15-4.8 greater risk in vertebral fractures (HR 3.15, 95% CI 1.35-6.18; HR 4.8, 95% CI 3.6-6.5) (225, 232), and 1.93-fold increase in non-vertebral fractures (HR 1.93, 95% CI 1.54-3.26) (232) in postmenopausal women.

In fact, osteoporosis and CVD share many common etiological factors, such as aging, menopause, dyslipidemia, smoking, alcohol consumption, and low physical activity (220). The two conditions also share common pathophysiological mechanisms, such as chronic low-grade inflammation and oxidative stress. Cross-sectional data suggest that adiponectin and leptin are related to markers of bone and vascular health in postmenopausal women and may contribute to the observed association between osteoporosis and CVD (214). Data from animal studies showed that leptin promotes osteogenic differentiation and vascular calcification (237). On the contrary, adiponectin reduces vascular calcification processes by either reducing cell apoptosis or attenuation of osteoblastic differentiation of vascular smooth muscle cells (238-240). However, the causal relationship between adiponectin and leptin with bone and cardiovascular health remains to be evaluated.

Although the nature of the putative link between osteoporosis and CVD remains unclear, vitamin D metabolism, hyperparathyroidism, and the receptor activator of RANKL/OPG pathway are implicated in the pathogenesis and progression of the two diseases (241). The

essential role of vitamin D for optimal calcium absorption and for bone mineralization is well established (242). Severe or prolonged vitamin D deficiency leads to rickets in children and osteomalacia in adults, and can exacerbate osteoporosis in older adults (243). Regarding the effect of vitamin D on cardiovascular health, observational studies have consistently associated vitamin D deficiency with increased risk for CVD and hypertension (244-249). However, a recent large scale trial (*n*=25,871) evaluated the effect of higher-dose vitamin D (2000 IU/day) on major cardiovascular events in men ≥50 years and women ≥55 years in the United States and vitamin D supplementation did not lead to a significantly lower incidence of a composite of major cardiovascular events (250). Further investigation with different doses of vitamin D is needed to confirm these observations, especially in vitamin D deficient individuals. Still, if vitamin D can reduce the incidence of major cardiovascular events, then the vasculo-protective properties of vitamin D may involve the activation of vasodilatory and antithrombotic gene programs (251, 252). In addition, vitamin D may also modulate the effects of inflammatory cytokines involved in bone metabolism and vascular integrity by suppressing the expression of multiple inflammatory cytokines (e.g., tumor necrosis factor alpha, IL-6, IL-1, and IL-8) (243). Taken together, this underlines the importance of assessing vitamin D status of participants in clinical trials with vascular health and bone health outcomes.

PTH is involved in age-related bone loss and bone fragility by acting as a key hormone regulating calcium homeostasis (253). Chronic hyperparathyroidism has been associated with hypertension, disturbances in the renin-angiotensin-aldosterone system, aortic stiffness, vascular calcification, increased CVD risk as well as increased cardiovascular mortality (220, 254-257). However, results of a Mendelian randomization study of individuals with a genetic predisposition to higher serum PTH concentrations did not show it being an independent risk

factor for CAD (258). These findings suggest that it is possible that the observed association between higher PTH concentrations with increased risk of CVD events could be a marker for another factor involved in the pathway leading to CVD events (256).

The RANK/RANKL/OPG axis is implicated in the pathogenesis of osteoporosis and CVD (259). OPG is a key regulator of bone resorption by serving as a decoy receptor for RANKL and inhibiting osteoclast activation (260). Low OPG levels have been associated with higher prevalence of osteoporosis and vertebral fractures in postmenopausal women (261). OPG also plays a role in vascular calcification, atherosclerosis, plaque destabilization, and CVD (262). Early-onset osteoporosis and calcification of the aorta and renal arteries were found in OPG-deficient mice (263). High levels of OPG have been positively related to severity of vascular damage, atherosclerosis progression and cardiovascular mortality, suggesting that elevated OPG levels may reflect a compensatory mechanism to prevent further vascular damage (255, 264, 265).

There has also been increasing interest about the role of the Wnt signaling pathway in the pathogenesis of vascular calcification (110). Wnt signaling plays a crucial role in bone metabolism and it is also associated with the progression of many diseases (110, 266). Cumulative evidence is suggestive that serum sclerostin may play a crucial role in the cross-talk between the vasculature and bone. Sclerostin, a key antagonist of the canonical Wnt signaling pathway and primarily secreted by mature osteocytes, regulates osteoblast activity and is a well-established key player in the regulation of bone homeostasis (267). Sclerostin reduces bone formation by inhibiting the differentiation of osteoblasts through the Wnt/β-catenin pathway (268) and stimulates bone resorption by stimulating osteoclast differentiation in a RANKL-dependent manner (269).

Cross-sectional observations have suggested a positive association between BMD and sclerostin levels in postmenopausal women and healthy adults (270-275). Interestingly, prospective data associated higher levels of serum sclerostin with higher fracture risk among postmenopausal women (268, 276). These observations were confirmed by findings from a Mendelian randomization study that found that higher sclerostin levels are causally related to lower BMD and greater fracture risk (277). Monoclonal sclerostin antibody, romosozumab, have been demonstrated to be an effective treatment of postmenopausal osteoporosis by increasing BMD and bone formation with decreased bone resorption in postmenopausal women with low bone mass (278). However, the anti-sclerostin treatment may increase cardiovascular risks as more serious cardiovascular adverse events (i.e., cardiac ischemic event, cerebrovascular event, heart failure, death, non-coronary revascularization, peripheral vascular ischemic event not requiring revascularization) were observed in the group that received romosozumab compared to those who received the comparative treatment alendronate (2.5% vs. 1.9%) (279). The higher cardiovascular event rates may be due to the potential cardio-protective effect of the comparison drug alendronate (279). Alternatively, the inhibition of sclerostin may lead to the activation of the Wnt signaling pathway and pathogenesis of atherosclerosis (280).

Sclerostin has been detected in calcifying vascular tissues in mouse models and hemodialysis patients (281-283). Cross-sectional data of ambulatory adults without chronic kidney disease consistently showed a positive association between serum sclerostin and cardiovascular measures including high blood pressure, cIMT, arterial stiffness, and aortic calcification (284-287). Furthermore, one prospective study showed that high serum sclerostin concentrations associated with increased risk of cardiovascular-related mortality (HR 1.32, 95% CI 1.09-1.60) (284). Recent findings from a study in mice with induced chronic kidney disease

suggest that increased sclerostin production may be a primary defensive response to mitigate ectopic calcification of soft tissues in the early stage of renal impairment (288). More specifically, renal impairment triggers local osteocytic production of 1,25-dihydroxyvitamin D which subsequently increases the production of sclerostin and the suppression of bone morphogenetic protein-2 to protect from further vascular calcification (288). Although more data in individuals without chronic kidney disease is required, the available evidence suggests that sclerostin may act as an independent key predictor of cardiovascular mortality, or increased circulating sclerostin concentrations may be a protective mechanism preventing further vascular damage.

In summary, vascular calcification and osteoporosis are both driven by inflammation and share similar mechanisms that involve biomarkers of mineral homeostasis. It may be hypothesized that calcium supplementation could increase vascular calcification in similar ways that it increases BMD (289, 290). There remains a knowledge gap in the literature regarding the potential pathways underlying the reported associations between bone markers with CVD. Future studies examining the effect of nutrition interventions on cardiovascular health should examine both inflammatory and bone health biomarkers to better understand the underlying mechanisms.

2.4.4 Association of calcium intake and inflammation

Few trials have investigated the effect of calcium supplementation with or without vitamin D supplementation on inflammatory markers. Studies reported no effect on pro-inflammatory cytokines or hsCRP following calcium supplementation interventions between 8 weeks and 1 year of duration (291-294). On the other hand, there is an increasing interest in the effect of milk and milk products on inflammation. Data from cross-sectional studies and interventional trials in healthy adults have generally shown no association or an inverse

relationship between consumption of milk and milk products and circulating inflammatory markers (22, 42, 44, 295, 296). Meta-analyses of short-term consumption (post-prandial response or 4-48 weeks) of dairy products in various forms and with various fat content have shown that milk and dairy products have no effect on systemic inflammation as measured by circulating biomarkers such as hsCRP, cytokines including interleukin-6, tumor necrosis factor-alpha and adiponectin (42, 297, 298). Similarly, evidence from an RCT with postmenopausal women found no significant effect of dairy foods on expression of inflammation-responsive or proteolytic genes (299).

Emerging evidence suggests that FMPs may provide more benefits than non-FMP on cardiometabolic health and bone health. This is in line with the growing interest and advances in research on the crosstalk between the gut microbiome and the different systems in the human body, including vascular and skeletal health. Researchers speculate that the probiotic content of FMPs may exhibit anti-inflammatory properties, resulting in a reduction in pro-inflammatory cytokines (300, 301). In fact, the probiotic bacteria found in cheese and yogurt have been shown to exert favorable effects on inflammation and cardiovascular risk factors (302). This warrants future research to evaluate the effect of each type of dairy product individually on health outcomes.

Given the lack of interventional studies comparing the effect of dietary calcium and supplemental calcium on the inflammatory biomarkers in healthy postmenopausal women, the analysis of these biomarkers will provide insight into the processes that may occur in the vasculature and whether the two sources of calcium have exert differential effects.

2.5 Assessment of calcium intake in epidemiological studies

2.5.1 Dietary assessment methods

There is currently no biologically valid biomarker of calcium intake. Therefore, epidemiological research relies on dietary questionnaires to estimate calcium intake. Dietary data can be collected by asking the respondent to recall what they had consumed the previous day (i.e., 24-hour recall), keep a detailed record of what they eat over one or more days (i.e., diet record), or by completing an FFQ to estimate usual intake over a longer period of time (303).

Among these methods, none of them is considered the 'gold standard'. Instead, the study design, its objectives, the target population and the resources available will determine which dietary assessment method is more suitable (304). For instance, a single food record or 24-hour recall would be appropriate to describe the average intake of a group of individuals (305). On the other hand, a one-day dietary intake data cannot be used to assess nutrient adequacy without some statistical applications (306). Furthermore, the estimation of usual intake of each specific nutrient requires a certain number of days of data for a given degree of accuracy. For calcium, data from a minimum of 13 days (e.g., 13 24-hour recalls or food records) are required to estimate a woman's calcium intake to ±20% of her true mean 95% of the time (307). Although 24-hour recalls and food records provide greater specificity for describing foods, these short-term methods require extensive effort and resources to estimate usual intake in epidemiological studies (308). These methods are commonly used to assess the validity of an FFQ, which is the preferred method for estimating usual intake in large-scale epidemiological research.

2.5.2 Food frequency questionnaires

FFQs are practical dietary assessment tools and present considerable advantages in terms of cost-effectiveness (309). FFQs can be qualitative or quantitative. A qualitative or simple FFQ

collects information on the frequency but no additional information on portion sizes. A semi-quantitative FFQ either specifies a portion size as part of the question on frequency, or includes an additional question about the usual portion size for each food item (310). Many FFQs have been developed and validated over the years, but due to cultural and geographic differences among population groups, the validity of the FFQ is limited to the populations for which the instrument was designed. Furthermore, the FFQ needs to reflect the current food supply and modifications to the existing questionnaire would be required. When no FFQ has been validated in the target population, then an FFQ can be designed de novo or researchers can use or modify an existing questionnaire (310). For example, no FFQ designed for epidemiological research has been validated in postmenopausal women living in Canada. The semi-quantitative FFQ used in the CaMOS has not been previously validated and can be easily adapted to use in Canadian postmenopausal women specifically (80, 311). This FFQ was also calcium-focused and required significantly less time to complete than questionnaires with an extensive list of items that aim to capture the usual intake of a variety of nutrients. A nutrient-focused FFQ may also reduce respondent burden and subsequently be an advantage to help minimize attrition rate and missing data. Lastly, alike other data collection methods, calcium intake estimated from an FFQ is prone to a certain degree of measurement error. Therefore, evaluating the relative validity of the FFQ against a reference method, such as multiple 24-hour recalls, can provide quantitative information that will help enhance the interpretation dietary data on health outcomes (312).

2.6 Summary

Current calcium intake of over 80% of Canadian postmenopausal women from dietary sources is below the recommendations of the EAR with an average estimated intake of 700 to 800 mg/day. Although many women have reported the use of calcium supplements, a clinical

equipoise exists due to uncertainties related to the effect of supplemental calcium on vascular health. Similarly, whether milk and milk products have pro-inflammatory or anti-inflammatory properties is unclear. This literature review highlighted that there is a lack of trials examining the effect of supplemental and dietary calcium on inflammatory markers in postmenopausal women and the mechanism by which calcium exerts its effect on the vasculature remains to be elucidated. Based on the proposed bi-directionality of osteoporosis and CVD, investigation into the effect of both dietary and supplemental calcium as compared to control on biomarkers of inflammation and bone health would allow a better understanding of the underlying mechanisms.

Bridge Statement 1

The literature review of this thesis indicates that the dietary calcium intake of approximately 80% of Canadian postmenopausal women remained below the EAR (1000 mg/day) over time (3, 67). Calcium intake from dietary sources is generally safe and adequate intakes are associated with higher BMD. However, very high intakes of milk have been associated with higher risk of all-cause and cardiovascular-related mortality in Swedish cohorts (84, 88), but these associations were not observed in other population groups (47, 80, 83).

Although calcium supplements are economical alternatives and allow users to compensate for the shortfall in the diet, meta-analyses suggested an association between supplemental calcium and increased risk of cardiovascular disease (8, 9). However, the proposed adverse cardiovascular effects have not been fully supported due to mixed findings across studies, and there remains a lack of clinical interventional studies with vascular health endpoints as primary outcomes.

Considering the high prevalence of osteoporosis and fragility fractures in older women, there is an urgency to understand the benefits and risks of dietary and supplemental calcium on extraskeletal health. No trials have been undertaken to compare the effect of dietary and supplemental calcium on vascular endpoints as primary outcomes prospectively.

The following study explored the feasibility of dietary interventions meeting the RDA (1200 mg/d) for calcium through dietary sources or predominantly from supplemental sources in healthy postmenopausal women ≥55 y. This pilot study gathered evidence in preparation for a larger RCT of similar design aimed to investigate the effect of supplemental and dietary calcium on vascular and bone health in healthy postmenopausal women.

CHAPTER 3

Manuscript 1: Feasibility Study

Published: *British Journal of Nutrition* 2016;116(1):104-14

Feasibility of a clinical trial to assess the effect of dietary calcium versus supplemental calcium on vascular and bone markers in healthy postmenopausal women

Angel M. Ong[1,2], Hope A. Weiler[2], Michelle Wall[1], Rouba Haddad[3], Jessica Gorgui[1], Stella S. Daskalopoulou[1,4], David Goltzman[1,4] and Suzanne N. Morin[1,4*]

Authors' Affiliation:

[1] McGill University Health Centre Research Institute, Montreal, QC H3G 1A4, Canada

[2] School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada

[3] Department of Nutrition, Shriners Children's Hospital, Montreal, QC H3G 1A6, Canada

[4] Department of Medicine, McGill University, Montreal, QC H4A 3J1, Canada

Abstract

Whether supplemental calcium has similar effects to dietary calcium on vascular and bone markers is unknown. The present trial investigated the feasibility of applying dietary and supplemental interventions in a randomised controlled trial (RCT) aiming to estimate the effect of supplemental calcium as compared to dietary calcium on vascular and bone markers in postmenopausal women. Thirteen participants were randomised to CaSuppl (750 mg calcium from $CaCO_3$ + 450 mg calcium from food + 20 μg vitamin D supplement) or CaDiet (1200 mg calcium from food + 10 μg vitamin D supplement). Participants were instructed on calcium consumption targets at baseline. Monthly telephone follow-ups were conducted to assess adherence to interventions (±20% of target total calcium) using the multiple-pass 24-hour recall method and reported pill count. Measurements of arterial stiffness, peripheral blood pressure, and body composition were performed at baseline, 6 and 12 months in all participants who completed the trial (n=9). Blood and serum biomarkers were measured at baseline and 12 months. Both groups were compliant to trial interventions (±20% of target total calcium intake; pill count ≥80%). CaSuppl participants maintained a significantly lower average dietary calcium intake compared to CaDiet participants throughout the trial (453 (SD 187) mg/d *v.* 1241 (SD 319) mg/d; P<0.001). There were no significant differences in selected vascular outcomes between intervention groups over time. Our pilot trial demonstrated the feasibility of conducting a large-scale RCT to estimate the differential effects of supplemental and dietary calcium on vascular and bone health in healthy postmenopausal women.

3.1 Introduction

Adequate intakes of calcium and vitamin D are essential for optimal bone health throughout adulthood to prevent osteoporosis and related fractures (5, 6, 313-316). Current Dietary Reference Intakes (DRIs) for calcium have been established by the Institute of Medicine for women over 50 years of age – that is, the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Level (UL) are 1000, 1200 and 2000 mg/d, respectively (58). However, adequate intake of calcium can be difficult to achieve through dietary sources alone (3, 4).

According to the NHANES (National Health and Nutrition Examination Survey) 2003-2006 data, less than 10% of American women over 50 years of age reached an intake of 1200 mg/d of calcium from food alone (4). Similarly, over 80% of Canadian women over 50 years of age had a calcium intake <1000 mg/d from dietary sources according to the 2004 Canadian Community Health Survey (CCHS) (3). Thus, to ensure adequate total calcium intake for skeletal integrity, calcium supplements are widely recommended (317, 318). Collectively, data from North America documented that 49-67% of women aged 50-70 years and 60-65% of those over 71 years old reported using supplements that contain calcium (3, 4).

Calcium supplementation is generally well tolerated but can be associated with mild gastrointestinal symptoms and with renal lithiasis (7). More recently, concerns about the use of supplemental calcium have been expressed following the publication of two meta-analyses, which reported that calcium supplementation, with or without vitamin D, increased the risk of cardiovascular events, in particular myocardial infarctions (8, 9). However, other analyses in similar populations have not reported this adverse association between calcium supplementation and cardiovascular events (10-13). On the other hand, most studies demonstrated that dietary

sources of calcium have not been linked with cardiovascular adverse events (8, 16, 17) or have been shown to be favorable to cardiovascular health (20-23). However, a recent report has raised uncertainty associating milk intake with a dose-dependent higher all-cause mortality rate, particularly cardiovascular death, in a large cohort of men and women over a 20-year period (40).

Cardiovascular disease is a leading cause of morbidity and mortality globally in women after the age of 50 years, with coronary artery disease and stroke representing 23% and 18% of cause of death, respectively (69, 70). In practice, early damage to the vascular system can be assessed non-invasively and accurately by measuring arterial stiffness, a composite indicator of vascular health that is an independent predictor of cardiovascular disease and events (319, 320). However, limited evidence exists on the effect of either supplemental or dietary calcium intake on arterial stiffness.

We conducted a 12-month pilot trial to determine the feasibility of controlled dietary and supplemental interventions of a randomised controlled trial that aims to assess the effect of supplemental calcium as compared to dietary calcium on arterial stiffness and other vascular and bone markers in postmenopausal women. The primary objectives were to test the feasibility of nutritional counselling on adherence to the dietary and supplemental interventions, and the tolerability of calcium and vitamin D supplementation. The secondary exploratory objective was to contribute preliminary evidence on the effect of dietary calcium versus supplemental calcium on vascular and bone health markers.

3.2 Methods

Study design and population

We conducted a randomised single-blinded parallel 1-year intervention trial from May 2012 to January 2014. Healthy postmenopausal women aged 55 years and older (≥3 years since

last menstrual period) without any chronic disease were recruited through posters and local newspaper advertisements in Montreal, Quebec, and were screened for eligibility by phone. Exclusion criteria were established to avoid including women with clinical or subclinical vascular impairment due to cardiovascular risk factors or cardiovascular disease. Specifically, postmenopausal women with lactose intolerance were excluded, as were those who smoked within the last 5 years, who had a body mass index (BMI) <20 kg/m^2 or >30 kg/m^2, history of diabetes, pre-eclampsia, hypertension, atrial fibrillation or atherosclerosis. Participants who had used hormonal replacement therapy (excluding vaginal preparations) in the last 3 years, medications to treat hypertension or hypercholesterolemia, or those known to affect bone metabolism (systemic glucocorticoids, bisphosphonates, receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor, selective estrogen receptor modulators, calcitonin, teriparatide) within the past 12 months were also excluded. Those with a 10-year absolute risk of major osteoporotic fractures >20%, computed using FRAX^{TM} without bone mineral density (BMD), were excluded as well (321). All participants were required to refrain from using nutritional supplements for 2 months prior to study entry. The present trial was conducted according to the guidelines laid down in the Declaration of Helsinki, and ethics approval was granted by the McGill University Health Centre Research Ethics Board. Written informed consent was obtained from all participants (GEN-11-231).

On-site visits occurred at baseline, and at 6 and 12 months after randomisation at the same research site. Participants were asked to abstain from vigorous physical activity and alcohol consumption for 48 hours and to fast overnight for 12 hours prior to their visit. Participants were randomised to one of two interventions on the day of the baseline visit. A web-based patient randomisation service (http://www.randomizer.at) was used to generate permuted block

randomisation in six-block intervals. The interventions were as follows: Calcium Supplement group (CaSuppl), 750 mg calcium carbonate supplement (Euro-Cal; Euro-Pharm International Canada Inc., Montreal, QC) + 450 mg from food sources + vitamin D supplement of 20 µg (800 IU) daily or Calcium Diet group (CaDiet), 1200 mg of calcium from food sources + vitamin D supplement of 10 µg (400 IU) daily. While very few foods naturally contain vitamin D, the CaDiet supplies 10 µg (400 IU) of dietary vitamin D from fluid milk and alternative plant-based beverages enriched with calcium, which are fortified with vitamin D in Canada.

Participants randomised to the CaSuppl arm were instructed by the research dietitian to limit their daily intake of dairy and alternative calcium-rich foods to 1 small portion (providing approximately 150 mg per serving). Participants randomised to the CaDiet arm were instructed to increase their intake of dairy and alternative calcium-rich foods to 3 portions a day (providing approximately 300 mg per serving). A total daily intake of 1200 mg of calcium, which included 300 mg of calcium from other foods in the diet, such as vegetables and grains (322), was obtained from the combination of supplementation and dietary sources in the CaSuppl group, and through dietary sources alone in the CaDiet group. Instructions on how to estimate portion sizes were provided at the baseline visit by the dietitian using food models and common household items. Educational tools including the Calcium Calculator™ by the British Columbia Dairy Foundation (323), the nutrition label reading handout by Health Canada (324), and sample menus were provided. Participants received monthly follow-up telephone calls to monitor health status, adverse events, and compliance to the assigned intervention.

Measurements

Measures of feasibility

The primary outcome measures for this pilot trial include participant adherence to the

trial protocol, and compliance to trial interventions, as well as tolerability of calcium and vitamin D supplementation. Compliance to trial interventions was defined as a mean total calcium intake ±20% of target (i.e. 1200 ± 240 mg), as per intervention assignment, and use of calcium supplements ≥80%. Tolerability of supplements was assessed by reported adverse events and acceptability of measurements was assessed by direct questioning by research personnel.

Questionnaires

Past medical history and family history of cardiovascular and bone disease were surveyed at the baseline visit, as well as use of medications and nutritional supplements. The International Physical Activity Questionnaire (IPAQ) (325, 326) and the Harvard-Willett Food Frequency Questionnaire (FFQ) (327) were administered at baseline and 12 months to estimate physical activity status and to determine nutrient intakes, respectively. Participants were asked to report the time they spent on specific types of physical activities in the last 7 days. Metabolic equivalents (METs) were calculated based on the activity type as per the IPAQ guidelines (328). A trained research member administered the FFQ, which was based on the participant's usual intake in the last 3 months. Multiple-pass 24-hour recalls (329) were administered at each on-site visit and at each monthly telephone follow-up. FFQ data were analysed using the Canadian Nutrient File (CNF) 2010b to calculate energy, calcium and vitamin D intake. The 24-hour recall data were analyzed using the Nutritionist Pro software (Axxya Systems, Stafford, TX) to calculate dietary calcium and vitamin D intakes and monitor adherence to the dietary interventions. Supplementation compliance was assessed by reported tablet count during monthly follow-up calls, and by verification of reported tablet count upon return of supplement bottles. General health status, use of new medications and nutritional supplements were also surveyed during monthly follow-ups.

Anthropometric measurements

Height, weight, waist and hip circumferences were measured at each visit using standard practices. Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 242), and weight was measured to the nearest 0.1 kg in light clothing without shoes (Tanita TBF-310; Tanita Corp.). Waist circumference was measured to the nearest 0.5 cm at the midpoint between the lower costal margin and the iliac crest according to Health Canada guidelines (330). Hip circumference was measured to the nearest 0.1 at the level of the symphysis pubis and the greatest gluteal protuberance based on the protocol followed by Health Canada, the *Canadian Standard Test of Fitness*, 3rd Edition (331).

Vascular measurements

Peripheral resting blood pressure was measured using the automated BpTRU device (BpTRU Medical Devices Inc.), according to the Canadian Hypertension Education Program guidelines (332). Arterial stiffness assessments were performed with the participant in a supine position after 10-minute rest in a quiet, temperature (20±1°C) and humidity (60±5%) controlled environment (Vascular Health Unit, McGill University Health Centre, Director: Dr. Daskalopoulou – co-author). Applanation tonometry (SphygmoCor, AtCor Medical) was used to measure carotid-femoral pulse wave velocity (cfPWV), the gold standard measurement assessing central arterial stiffness, as well as carotid-radial PWV (crPWV), which is a measure of peripheral stiffness. Specifically, cfPWV and crPWV were measured in triplicates (and averaged out) using a high-fidelity micromanometer on the tip of a hand-held tonometer (SPC-301; Millar Instruments), which was applied over the carotid and femoral (or radial) arteries, and a 3-lead electrocardiogram. To minimize variability between replicate measurements, we discarded PWV measures that had consecutive readings with a difference that was >0.5 m/s (333). PWV

measures with individual-site (carotid or femoral) electrocardiogram (ECG)-pulse transit time standard deviations greater than 5% and overall standard deviation greater than 10% were discarded and the measurement was repeated. PWV with a heart rate difference between the carotid and femoral site of more than or equal to 5 beats per minute were also rejected, and the measurement was repeated. Only high-quality measurements were accepted. After measuring the distance between the recording sites, the PWV was calculated [PWV=distance (m)/transit time (s)] (334-338). To minimize the effect of the circadian cycle, assessments were performed at the same time in the morning at each visit (see **Supplementary Table 3.1**).

Biomarkers

Fasting blood samples were collected between 7.00 and 10.00 hours at baseline and final visits to minimize any variation owing to biological rhythms of most biomarkers. Ionized calcium was analysed within 30 minutes of sampling at the Division of Biochemistry at the Montreal General Hospital (MGH) with the ABL 800 series blood gas analyzer (Radiometer America), with an intra-individual % CV of 2.8% based on internal quality controls for ionized calcium. Remaining blood samples were separated into plasma and serum fractions, and stored at -80°C until further analysis. Measurements of total cholesterol, high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TAG), apolipoproteins A1 (apo-A1) and B (apo-B), and high sensitivity C-reactive protein (hsCRP) were performed with a Synchron LX Systems (Beckman Coulter Inc., California, USA) at the end of the trial period in one single batch at the MGH. The intra-assay % CV was 0.7% for total cholesterol, 0.6% for TAG, 1.5% for HDL-c, 0.9% for apo-A1, 1% for apo-B, 0.9% for hsCRP. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (339). Plasma total 25-hydroxy vitamin D (25(OH)D) and PTH concentrations were measured using an autoanalyzer (Liaison, DiaSorin,

Ontario, Canada) with an intra-assay coefficient of variation of 4.1% and 3.6%, respectively. All 25(OH)D and PTH measurements were performed at the School of Dietetics and Human Nutrition of McGill University, which participates in the international Vitamin D External Quality Assessment Program (http://www.deqas.org) and obtained a certificate of proficiency for 2011-2015 (Director: Dr. Weiler – co-author). Accuracy of the methodology for vitamin D assays was assessed by the use of the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM 972a, Level 1 and Level 2). Accuracy of Liaison controls were 94.4-100.4% for 25(OH)D and 96.5-97.8% for PTH.

Online feedback survey

Following completion of this pilot trial, all participants were invited to complete an online feedback survey "Participation in Pilot Calcium Study: Feedback Survey" anonymously using the Lime Survey application (http://www.limesurvey.org). The survey aimed to evaluate the acceptability of the trial interventions, participant satisfaction with the dietary modifications, supplement regimen, trial visits and procedures, as well as monthly contacts with the dietitian. The survey included an open-ended question for the participants' feedback on the strengths and limitations of the trial design.

Statistical analyses

Summary statistics were computed for baseline characteristics, presented as mean and standard deviation for normally distributed continuous variables, or count and percentage for categorical variables. Chi-squared test was used to test for differences in proportions. Independent student *t*-tests were used to compare the differences in mean intakes of dietary calcium and vitamin D at 1-6 months, 7-12 months, and at 12-months between the two intervention groups. Independent student *t*-tests were also used to compare the differences in

blood pressure, anthropometric measurements, physical activity, vascular and bone biomarkers at 12 months and over time. Within and between-group differences over time in cfPWV were examined using repeated measures ANOVA, as per intention-to-treat analysis. Due to the exploratory nature of the trial and small sample size, no multiple-testing correction was performed (340). Significance was set at $P<0.05$. All statistical analyses were performed using the statistical software package SPSS version 22 for Windows (SPSS Inc.).

3.3 Results

The Consolidated Standards of Reporting Trials (CONSORT) flowchart depicting participant selection process is demonstrated in **Figure 3.1**. Thirteen women were enrolled in the trial between July 2012 and February 2013 at a mean age of 63.2 (SD 6.6) years, BMI of 24.6 (SD 2.9) kg/m^2, systolic blood pressure 111.1 (SD 13.0) mmHg, and diastolic blood pressure of 71.3 (SD 10.1) mmHg. Seven participants were randomised to the CaSuppl group and 6 participants to the CaDiet group. In total, 3 participants withdrew from the trial and 1 was lost to follow-up. Dropout rates were similar between groups. Of the 4 participants who did not complete the trial, 1 withdrew from the trial when her family doctor initiated her on anti-hypertensive medication within 2 months following randomisation; her blood pressure at randomisation was within normal limits. We were unable to ascertain her blood pressure as she did not return for on-site visits and we were unsuccessful in contacting her physician.

No differences in baseline characteristics were observed between the two groups in age, anthropometric variables, physical activity level, nutrient intake including calcium and vitamin D, or vascular variables (**Table 3.1**). The only statistically significant difference between groups was a higher reported family history of stroke in the CaSuppl group ($P=0.03$).

In all, nine participants completed all telephone follow-ups and on-site visits. Dietary

data derived from the multiple-pass 24-hour recalls indicated that both the CaSuppl group and the CaDiet group participants were within 20% of their calculated calcium intake target over the trial period (94-113% and 96-116%, respectively). The calculated average dietary calcium intake from the multiple-pass 24-hour recalls indicated that the CaSuppl group had significantly lower mean intakes (480 (SD 292) mg/d at 1 month, 400 (SD) mg/d at 6 months and 600 (SD 93) mg/d at 12 months) than the CaDiet group (1380 (SD 437) mg/d at 1 month, 1269 (SD 188) mg/d at 6-months and 1019 (SD 323) mg/d at 12 months) ($P<0.05$, **Figure 3.2**). During the 12-month intervention, the CaSuppl group had a significantly lower intake of dietary vitamin D at 6 months (1.54 *v*. 11.8 µg) ($P<0.05$). The 12-month average total calcium intake (dietary + supplemental calcium) in the CaSuppl group was 1124 mg/d *v*. 1242 mg/d in the CaDiet and we found no significant differences ($P=0.13$). When we compared the usual dietary calcium intake data derived from the Harvard-Willett FFQ, there was a significant between group difference at 12-months ($P=0.04$). However, the mean dietary calcium intake was 761 (SD 277) mg/d in the CaSuppl group and 1187 (SD 232) mg/d in the CaDiet group. Mean dietary vitamin D intakes derived from the FFQ method at 12-months were 3.42 (SD 1.50) µg/d and 10.43 (SD 4.28) µg/d in the CaSuppl and CaDiet groups, respectively. Overall adherence rate to calcium supplements was 85% in the CaSuppl group and adherence rate to vitamin D supplements was 98% in both groups. None of the participants reported adverse events from supplements.

We found no significant differences in body weight, BMI, waist circumference, waist-to-hip ratio or physical activity level between the intervention groups at 12 months and no significant within-group change over time was observed ($P>0.05$).

Following the 12-month intervention, no significant differences in blood pressure or vascular health biomarkers were found between groups or within-groups over time (**Table 3.2**).

We observed that the systolic blood pressure was higher in the CaSuppl group compared to the CaDiet group at 12 months, though not reaching statistical significance (P=0.05). Systolic blood pressure values were normal in both groups at 12 months. Similarly, we observed a mean increase of 8.6 mmHg in diastolic blood pressure in the CaSuppl group *v*. a mean increase of 1 mmHg in the CaDiet group, but the between group change over time was not statistically significant (P=0.07); diastolic blood pressure values were normal in both groups at 12 months. Arterial stiffness measurements were completed with a mean duration of less than 45 minutes with each participant at each visit without adverse reactions. No between-group differences in cfPWV or crPWV were observed over time (**Figure 3.3**). A significant increase in crPWV was observed between 6 and 12 months in the CaDiet group (P=0.03).

Plasma lipid concentrations did not differ significantly between the CaSuppl and CaDiet following the 12-month intervention (**Table 3.2**). No significant difference in change over time between groups was observed with the exception of HDL-C, for which there was an increase of 0.20 mmol/l in CaSuppl and a decrease of 0.15 mmol/l in CaDiet (P=0.02). Levels of apo-A1, apo-B, and hsCRP did not differ significantly between intervention groups. No between group differences at 12 months were observed in markers of bone health, including ionized calcium, 25(OH)D, PTH, and phosphate. There was a decrease in ionized calcium of 0.03 mmol/l (P=0.007) and an increase of 0.57 pmol/l in PTH (P<0.001) between baseline and end of trial in the CaDiet. A mean decrease of 3.70 nmol/l in 25(OH)D level was also observed in the CaDiet group, but this was not statistically significant (P= 0.33) (**Table 3.3**).

Responses from the online survey (n=8) showed that all participants found the adherence to daily supplementation easy. While only 25% of the CaDiet group reported having difficulty meeting the daily dietary calcium target of 3 portions of dairy and alternatives, all of the

participants from CaSuppl group found it challenging to adhere to the dietary restriction of 1 small portion of calcium-rich food a day. Reasons provided by CaSuppl participants encountering difficulties included "*not being able to eat some favorite foods*" or had to "*reduce some of the regularly consumed foods significantly*". One participant felt that "*it was difficult to measure some foods*" to adequately report the portion consumed during telephone follow-ups. Overall, all participants felt adequately informed on how to modify their intake of dietary calcium from the baseline nutritional education session and the monthly follow-ups.

3.4 Discussion

Our pilot trial demonstrated the feasibility of conducting a large-scale randomised controlled trial to estimate the effect of supplemental calcium as compared to dietary calcium on vascular and bone health in postmenopausal women. In particular, we demonstrated that the combination of an initial in-person nutritional counselling session and monthly telephone follow-ups was effective to ensure participant adherence to the dietary aspects of the trial protocol. Participants felt that the monthly contacts with the dietitian were efficient to help them maintain the proper dietary calcium intake throughout the trial. In addition, we were able to demonstrate the feasibility in conducting the visits and comprehensive set of vascular tests without adverse reactions or unforeseen problems.

Preliminary results from this pilot trial did not show any differential effects of calcium supplementation as compared to dietary calcium on arterial stiffness nor on vascular health biomarkers. There was a significant increase in crPWV from 6 to 12 months in the CaDiet group. These observations may suggest an increase in peripheral stiffness, but have no clinical significance on aortic stiffness, which is the gold standard of arterial stiffness (341). It is noteworthy that the values of vascular health markers (cfPWV and blood pressure) were well

within normal limits in our study group, at all time points (342, 343). However, the results have to be interpreted with caution given the small sample size.

The rigorous design of our trial differentiates it from other randomised controlled trials that investigated the effect of calcium supplements on vascular and bone health. None of the previous trials controlled for dietary intake of calcium despite the reported administration of 1000 to 1200 mg/d of elemental calcium to treatment groups (11, 98, 316, 344, 345). In our trial, a dietitian evaluated baseline calcium and vitamin D intake and provided early nutritional education to each participant at the initiation of the trial to ensure a total daily intake of 1200 mg calcium either from dietary sources alone or predominantly from calcium supplements. The regular monthly follow-ups allowed close monitoring of protocol adherence and further counseling to ensure adherence to the trial protocol. We observed a high compliance of 85% to calcium supplements, while comparable trials reported compliance rates between 59% and 85% (11, 98, 316, 344, 345). We also observed that the rigorous design of our pilot trial was effective in helping achieve the target dietary calcium in each group at monthly follow-ups. However, the CaSuppl average dietary calcium intake estimated from the Harvard-Willett FFQ at 12-months was above the assigned calcium target (761 *v.* 450 mg/d). This observed difference may be a result of over-reporting by one participant during the final visit. Given the small sample size, the mean value is expected to be influenced by one single result. Furthermore, a calcium-focused FFQ may likely be less challenging conceptually for the participant, reduce the respondent burden, and increase the accuracy of dietary calcium intake. Milk and dairy products were most often consumed by the participants in this feasibility trial to meet their intervention targets. Although the Harvard-Willett FFQ includes a section to assess the intake of milk and dairy products, it does not discriminate soft or semi-soft cheeses from firm or hard cheeses. Similarly,

this semi-quantitative FFQ does not include calcium-rich items that are currently commonly consumed, such as Greek yogurts, beverages enriched with calcium, canned salmon with bones, pizza, and mixed dishes prepared with milk or dairy products. Given the popular consumption of these listed items by the participants in our feasibility trial, a short comprehensive calcium-focused semi-quantitative FFQ may improve the estimation of usual dietary calcium intake, and likely decrease respondent burden.

Thus far, observational and ancillary studies have investigated the possible relationship between calcium supplements and cardiovascular events following the publication of a meta-analysis of calcium intervention trials (8) and a secondary analysis of the Women's Health Initiative (WHI) calcium/vitamin D supplementation study's dataset (9). More specifically, Bolland et al. demonstrated an increased risk of myocardial infarction in individuals who received calcium supplements alone (8) (hazard ratio [HR] 1.31, 95% CI 1.02-1.67) or with vitamin D (9) (HR 1.21, 95% CI 1.01-1.44) based on self-reported and verified myocardial infarctions. In contrast, a recent meta-analysis of published and unpublished data from randomised controlled trials of women only by Lewis et al. demonstrated an absence of increased risk of coronary heart disease (RR 1.02, 95% CI 0.96-1.09) or all-cause mortality (relative risk [RR] 0.96, 95% CI 0.91-1.02) from calcium supplements with or without vitamin D in elderly women (14). Yet, the evidence remains conflicting, which may be due to the primary investigated endpoint of randomised controlled trials not being related to cardiovascular risks and an oversight on the assessment of dietary calcium intakes (12-14, 102, 346).

Observational studies suggest an association between higher serum calcium concentrations and carotid artery plaque thickness (347), increased risk of myocardial infarction and stroke (348) in older men and women. An increase in total serum calcium level of 0.1

mmol/l has been reported to be associated with 23% higher odds of abdominal aortic calcification in postmenopausal women (349). Furthermore, it has been previously shown that ionized calcium concentrations increase acutely following supplementation with 1000 mg of calcium supplement (350, 351), though whether these observed changes are maintained with chronic calcium supplement use is unknown. Reid et al. speculate that the increase in serum ionized calcium concentrations may lead to a sequence of events contributing to the acceleration of vascular disease by calcium supplementation. They hypothesised that the pathogenic pathway leading to progressive arterial calcification is via a loss of inhibition of mineralisation due to increased complexing of ionized calcium with pyrophosphate (101), reduced inhibition of arteriosclerotic signaling in vascular smooth muscle cells due to decreased PTH levels (102), and increased binding to calcium-sensing receptors on vascular smooth muscle cells and platelets (101). Increased arterial stiffness and impaired endothelial vasodilator function resulting from high circulating levels of ionized calcium associated with calcium supplementation could also lead to vascular damage (102). However, Burt et al. examined the acute effect of 1000 mg of calcium citrate on arterial stiffness in adults 50 years of age and older and found no significant change in PWV 3 hours following supplementation (351).

Arterial stiffness, as measured by the 'gold-standard' cfPWV, is an overall indicator of vascular health and is strongly associated with the development of atherosclerosis at different sites of the arterial system and cardiovascular disease and events (319, 320, 352, 353). An increase in cfPWV by 1 m/s corresponds to an adjusted risk increase of 14%, 15% and 15% in total cardiovascular events, cardiovascular mortality, and all-cause mortality, respectively (352). Using this gold standard measure, dairy food intake has been shown to have a favorable effect on arterial stiffness, as well as overall cardiovascular profile and reduced mortality (74, 75, 77, 354).

However, the evidence regarding the effect of calcium in the form of supplements on arterial stiffness is less consistent. When administered to healthy volunteers, calcium citrate (single oral dose of 1000 mg, measurements at times 0, 60, 120 and 180 minutes) was observed to cause an acute increase in total and ionized calcium (by an average of 0.10 and 0.06 mmol/l, respectively) and a decrease in PTH (351). However, the acute increase in calcium was associated with reduced arterial wave reflection, which in the long-term may reduce cardiovascular risk (352). In an acute loading cross-over trial, 600 mg of calcium citrate and 600 mg of calcium from dairy products did not produce any differential effect on arterial stiffness in young healthy subjects 2 h after each challenge (355). In our pilot trial, calcium supplements did not affect blood pressure, cfPWV, or crPWV differently than dietary calcium throughout the 12-month intervention.

The effect of calcium supplementation on serum lipids is inconsistent in the literature. Some studies report that supplemental calcium may cause beneficial changes in circulating lipids (356, 357), whereas several studies found no effect (358-361). In contrast, calcium supplementation as compared to placebo for 12 months improved the lipid profile in a randomised controlled trial of postmenopausal women, and this improvement was still observed at the 3-year follow-up (362). Dairy foods have been reported to have either neutral or beneficial effects on blood lipid profile (29-32). In contrast, supplemental calcium in the form of calcium carbonate did not exert such an effect on blood lipid concentrations (361). Herein, we observed an increase in HDL-C in the supplement intervention group but no other change in blood lipid profile. An increase in HDL-C concentration following calcium supplementation has been reported in the literature (356, 363, 364), and may likely be a result of the complexing of fatty and bile acids by calcium in the intestinal tract (356, 365).

Although our trial provides enough information to ascertain the feasibility of a larger randomised controlled trial, it is limited by its small sample size and therefore prevents conclusions regarding effects of the calcium interventions on vascular parameters. Although we experienced a dropout rate of 30% (4/13 participants), in keeping with other randomised controlled trials of calcium interventions (344, 362, 366), there were no differential dropout rates between the trial groups. Motivational interviewing techniques and participant satisfaction should be emphasized immediately following intervention initiation to improve participant retention as this pilot trial shows that the first months were critical times for dropout. An adequately powered clinical trial is warranted to effectively elucidate the effect of supplemental calcium on vascular health in postmenopausal women, and is currently underway by our group (ClinicalTrials.gov number, NCT01731340). Given that we have modified the procedures based on the online feedback survey of participants after the completion of the pilot trial, we anticipate a lower dropout rate in our ongoing randomised controlled trial.

In conclusion, the findings of this 12-month pilot trial indicate that both diet modifications and supplemental interventions were associated with high compliance and tolerance, demonstrating the feasibility of the interventions. Although limited by a small sample size, the results suggest that following this 12-month intervention, supplemental calcium does not exert a different effect than dietary calcium on vascular or bone health markers in healthy postmenopausal women. Nevertheless, the current state of uncertainty warrants further research to assess the effect of supplemental versus dietary calcium on the development of cardiovascular disease and whether this effect is mediated through established cardiovascular markers. In this context, results of an adequately powered randomised controlled trial will facilitate the development of public health recommendations regarding calcium supplementation. Building on

our findings of the feasibility of the trial interventions, a randomised controlled trial is currently ongoing by our group.

Table 3.1 Baseline characteristics of thirteen participants by intervention group (Mean values with standard deviations; number of participants and percentages)

Characteristics	CaSuppl (*n*=7)		CaDiet (*n*=6)		
	Mean	**SD**	**Mean**	**SD**	***P***
Age (years)	65.0	4.2	60.8	8.5	0.26
Caucasian (*n*, %)	4 (57.1)		5 (83.3)		0.31
Menarche age (years)	13.0	1.4	12.5	1.87	0.59
Menopause age (years)	49.3	5.8	47.5	6.8	0.62
BMI (kg/m^2)	24.6	2.8	24.6	3.33	0.10
Waist circumference (cm)	80.8	8.4	81.7	8.11	0.85
Hip circumference (cm)	97.8	7.9	98.6	2.4	0.80
Waist to Hip ratio	0.83	0.03	0.83	0.07	0.98
Blood pressure					
Systolic (mmHg)	114	16	108	8	0.38
Diastolic (mmHg)	70	13	73	5	0.71
Arterial stiffness					
cfPWV (m/s)	7.68	1.4	7.87	2.2	0.85
crPWV (m/s)	7.67	0.8	8.16	1.4	0.43
iCalcium (mmol/l)	1.26	0.3	1.27	0.3	0.56
25(OH)D (nmol/l)	60.2	25.2	63.4	36.0	0.85
PTH (pmol/l)	3.30	1.18	2.62	1.19	0.33
Family history (*n*, %)					
Osteoporosis	4 (57.1)		2 (33.3)		0.39
Coronary artery disease	4 (57.1)		2 (33.3)		0.39
Stroke	4 (57.1)		0		0.03
Dyslipidemia	3 (42.9)		5 (83.3)		0.14
Hypertension	3 (42.9)		5 (83.3)		0.14
Diabetes	3 (42.9)		2 (33.3)		0.73
Physical activity (MET-min/week)	3320	1502	6044	6150	0.33
Dietary intake (per d)*					
Energy (kJ)	10496	2717	9471	2378	0.49
Calcium (mg)	1054	317	1170	127	0.42
Vitamin D (µg)	15.6	10.2	12.3	2.22	0.47

CaSuppl, Calcium Supplement group; CaDiet, Calcium Diet Group; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; iCalcium, ionized calcium; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; MET-min/week, Metabolic Equivalent minutes per week.
*Dietary data derived from the Harvard-Willett semi-quantitative food frequency questionnaire.

Table 3.2 Changes in blood pressure and vascular health biomarkers from baseline to the end of the trial (12 months) (Mean values and standard deviations)

	CaSuppl (*n*=5)				CaDiet (*n*=4)					
	Baseline		12 months		Baseline		12 months		*P* (CaSuppl v. CaDiet at 12 mo)	*P* (ΔCaSuppl v. ΔCaDiet)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
cfPWV (m/s)	8.25	1.09	8.70	1.80	8.71	2.08	7.30	1.10	0.24	0.16
crPWV (m/s)	7.45	0.82	7.60	0.90	8.55	1.45	8.60	0.90	0.13	0.93
SBP (mmHg)	113.6	18.8	125.0	15.0	103.8	6.8	104.0	9.0	0.05	0.10
DPB (mmHg)	67.4	14.1	76.0	14.0	70.8	5.1	70.0	6.0	0.46	0.07
Cholesterol (mmol/l)	5.39	1.02	5.78	0.51	6.14	0.38	6.10	0.84	0.50	0.38
TAG (mmol/l)	0.93	0.56	1.22	0.87	0.88	0.28	0.80	0.38	0.40	0.20
HDL-c (mmol/l)	1.78	0.77	1.98	0.81	1.99	0.53	1.84	0.41	0.76	0.02
LDL-c (mmol/l)	3.19	0.98	3.25	0.61	3.00	1.95	3.90	1.04	0.28	0.27
Apo A1 (g/l)	1.31	0.75	1.76	0.33	1.65	0.35	1.59	0.28	0.45	0.11
Apo B (g/l)	1.04	0.33	1.07	0.26	1.17	0.24	1.20	0.32	0.55	1.00
hsCRP (mg/l)	1.68	1.71	0.76	0.49	0.33	0.15	0.38	0.24	0.19	0.21

cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; TAG, triacylglycerol, HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; Apo, apolipoprotein; hsCRP, high-sentivity C-reactive protein
Δ before and after intervention comparisons

Table 3.3 Changes in ionized calcium, 25(OH)D, and PTH from baseline to the end of the trial (12 months) (Mean values and standard deviations)

	CaSuppl (*n*=5)				CaDiet (*n*=4)					
	Baseline		12 months		Baseline		12 months		*P* (CaSuppl v. CaDiet at 12 mo)	*P* (ΔCaSuppl v. ΔCaDiet)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
iCalcium (mmol/l)	1.25	0.04	1.26	0.03	1.28	0.03	1.25	0.03	0.65	0.06
25(OH)D (nmol/l)	54.1	26.6	63.6	13.9	82.8	22.0	79.1	25.4	0.28	0.23
PTH (pmol/l)	3.50	1.33	2.89	0.72	2.27	0.29	2.84	0.29	0.93	0.06
Phosphate (mmol/l)	1.30	0.16	1.27	0.11	1.23	0.26	1.29	0.17	0.86	0.49

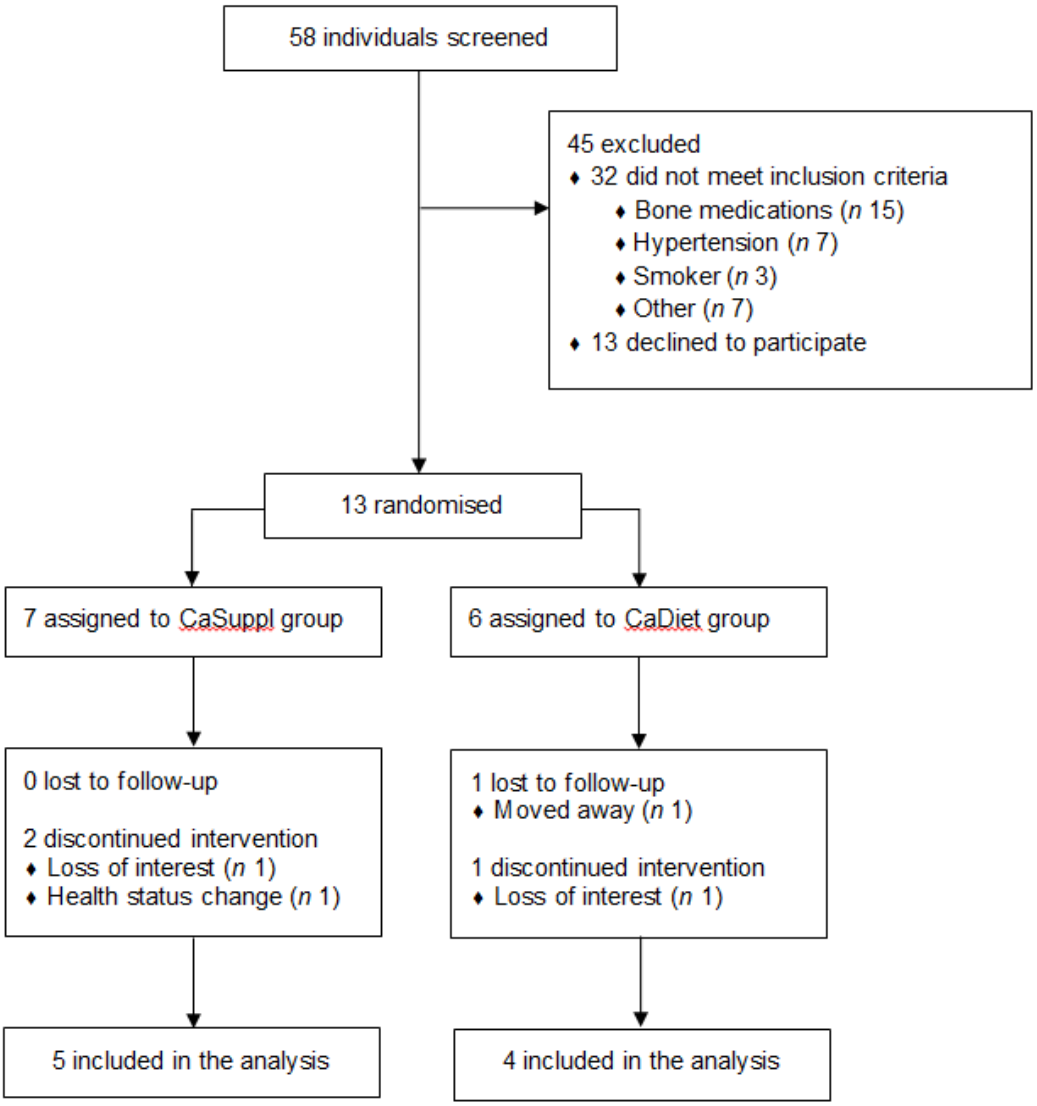
iCalcium, ionized calcium; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
Δ, Before and after intervention comparisons

Supplementary Table 3.1 Methods: schedule of visit and follow-up assessments*

	Baseline	1- to 5-month†	6-month	7- to 11-month†	12-month	Post-trial
On-site visit	●		●		●	
Telephone follow-up		●		●		
Dietary education	●	●	●	●	●	
Questionnaires						
Medical history	●					
IPAQ	●				●	
FFQ	●				●	
24-hour recall	●	●	●	●	●	
General health status		●	●	●	●	
Feedback survey						●
Anthropometry	●		●		●	
Vascular assessments						
Blood pressure	●		●		●	
Arterial stiffness	●		●		●	
Blood samples	●				●	

IPAQ, International Physical Activity Questionnaire; FFQ, food frequency questionnaire.
*See methods for details of the procedures
†Assessments were completed at monthly intervals.

Figure 3.1 Consolidated Standards of Reporting Trials (CONSORT) Diagram depicting the flow of participants.



(a) (b) (c)

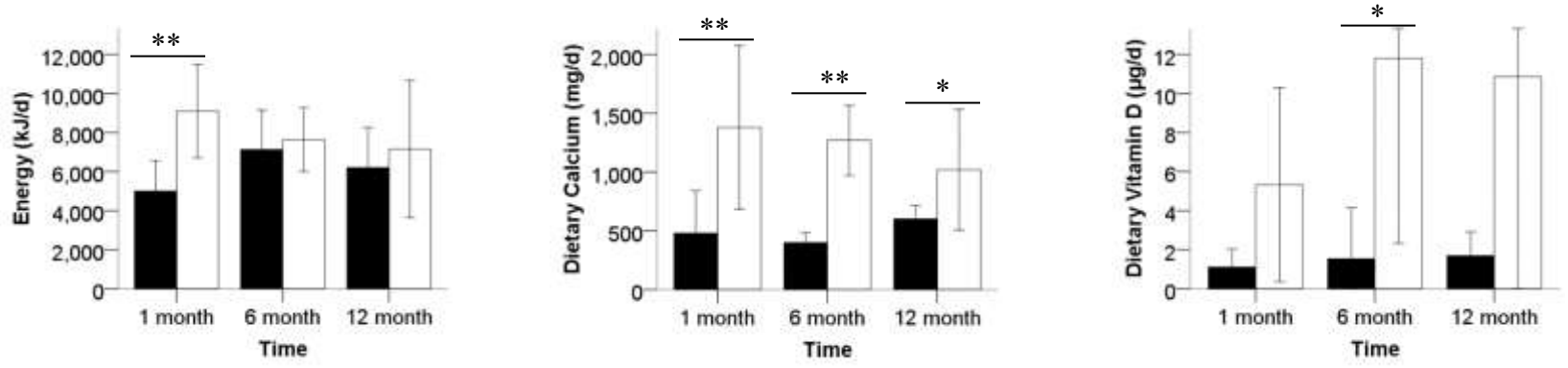


Figure 3.2 Mean energy (a), dietary calcium (b) and dietary vitamin D (c) intake over time. Values are shown as means, with 95% CI represented by vertical bars. Mean value was significantly different from that of the CaDiet: * $P<0.05$, ** $P<0.01$. ■, CaSuppl; □, CaDiet.

(a) (b)

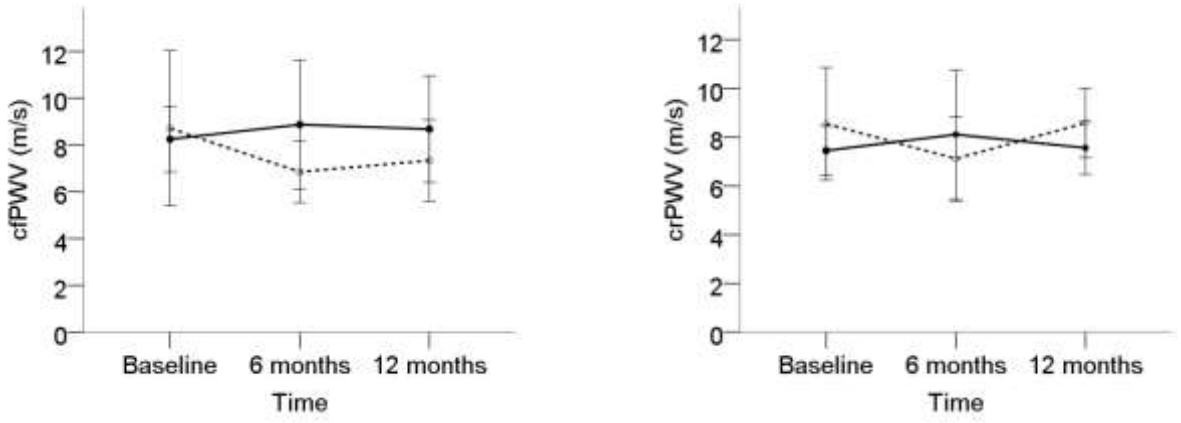


Figure 3.3 Change in arterial stiffness markers, (a) carotid-femoral pulse wave velocity (cfPWV) ($P_{timexintervention}$ = 0.16) and (b) carotid-radial pulse wave velocity (crPWV) ($P_{timexintervention}$ = 0.93), over time. Data shown as mean and 95% CI. ▬▬ , CaSuppl; ▬ ▬ ▬, CaDiet.

Bridge Statement 2

In Chapter 3, results of the 12-month feasibility study demonstrated high adherence and tolerance to both dietary modification and supplemental interventions. These results confirm the feasibility to conduct a larger scale RCT of a similar design in healthy postmenopausal women. However, the FFQ used in the feasibility study was limited in the range of milk and milk products available, such that it did not discriminate different types of cheeses with varying calcium content. The FFQ also did not adequately reflect the current food supply of calcium-rich foods in Canada, which reduce the accuracy of the estimated calcium intakes. As described in Chapter 2, FFQs need to be population-specific with regards to their geographical and cultural characteristics as well as the food supply to more accurately interpret the relationship between the nutrient intakes and health outcomes.

Therefore, Chapter 4 aimed to assess the relative validity of a 51-item semi-quantitative FFQ to estimate dietary calcium intake among postmenopausal women living in the Greater area of Montreal, Quebec, Canada. This tool was designed for use in the main RCT described in Chapter 6.

CHAPTER 4

Manuscript 2: Development and Validation of a Calcium-Focused Food Frequency Questionnaire

Published: *Nutrition Research* 2017;43:33-42

A 51-item calcium-focused food frequency questionnaire is a reliable tool to assess dietary calcium intake in postmenopausal women

Angel M. Ong[a,b], Hope A. Weiler[b], Michelle Wall[a], David Goltzman[c,d], Susan J. Whiting[e], Stella S. Daskalopoulou[a,c], Suzanne N. Morin[a,c]

Author Affiliations:

[a] Division of General Internal Medicine, Research Institute of McGill University Health Centre, Montreal, QC, Canada H3G 1A4

[b] School of Dietetics and Human Nutrition, McGill University, Ste. Anne de Bellevue, QC, Canada, H9X 3V9

[c] Department of Medicine, McGill University, Montreal, QC, Canada H3G 1Y6

[d] Division of Endocrinology and Metabolism, Research Institute of the McGill University Health Centre, Montreal, QC, Canada, H4A 3J1

[e] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada, S7N 2Z4

Abstract

Given the lack in a valid biomarker to assess dietary calcium intake (dCa_i), reproducible estimation of usual dCa_i is crucial for better understanding of its interaction with health outcomes in specific populations. This study tested the hypothesis that a calcium-focused food frequency questionnaire (FFQ) may be used to estimate dCa_i of women ≥50 years residing in a multicultural environment (Montreal, Canada). One hundred and eight women (age, 63.1±7.7 years; 98% postmenopausal) completed the FFQ twice and 4 nonconsecutive 24-hour recalls (24HRs) over 1 month. Medians of dCa_i were compared by Wilcoxon signed rank test. Reproducibility and relative validity of the FFQ were assessed by Spearman correlation (r_s) and Cohen's weighted kappa (κw). Agreement was further assessed by cross-classification by quartiles, Bland-Altman plot, and sensitivity and specificity analyses. The median (interquartile range) dCa_i estimated by the FFQ and 24HRs were 723 (524-1033) mg/d and 854 (666-1068) mg/d, respectively (P<0.001). The FFQs had a strong correlation (r_s=0.72, P<0.001) and moderate agreement (κw=0.55). The FFQ and 24HRs were moderately correlated (r_s=0.65, P<0.001). Cross-classification showed moderate agreement (κw=0.42), with 85% of the participants classified into identical or contiguous quartiles and 2.8% into extreme opposite quartiles. According to the Bland-Altman plot, the FFQ underestimated dCa_i with a bias of 99 mg/d (95% limits of agreement, -677 to +480 mg/d). Sensitivity and specificity of identifying intakes <1000 mg/d were 90% and 57%, respectively. This FFQ is a useful tool to discriminate dCa_i <600 and ≥1000 mg/d in postmenopausal women, and to rank dCa_i in epidemiological studies.

4.1 Introduction

The benefits of adequate calcium and vitamin D intakes, from dietary and supplemental sources, in the prevention and management of osteoporosis have been established (6, 18, 313, 314, 317, 367). In addition to its role for optimizing skeletal health, sufficient calcium intake is also associated with reduced risks of developing chronic diseases such as hypertension and colon cancer (368, 369). Current Dietary Reference Intakes established by the Institute of Medicine for calcium for women 51 through 70 years of age include the Estimated Average Requirement (EAR) (1000 mg/d) and the Recommended Dietary Allowance (1200 mg/d) (370). In Canada, mandatory fortification of foods with calcium for adults is limited to meal replacement products and nutritional supplements (371). Under the Food and Drugs Act of Health Canada, voluntary fortification with calcium is permitted for some foods, including flour, plant-based beverages (e.g., soy beverage), and orange juice (371, 372). Although plant-based beverages and orange juice fortified with calcium are calcium-rich foods, major sources of dietary calcium in the Canadian diet consist mainly of dairy products including milk, cheese and yogurt (3). Calcium is also present in large amounts in some other nondairy foods including canned salmon with bones, tofu prepared with calcium, and dark leafy greens (373).

Accurate assessment of calcium intake from dietary sources is used to determine an individual's supplemental requirements in clinical practice and in population surveillance. According to the 2004 Canadian Community Health Survey, more than 80% of women 50-70 years of age did not meet the calcium EAR from their dietary intake, and approximately a third of those who used supplements had a total calcium intake below the EAR (3). The adjusted average intake was estimated from 1 single 24-hour recall (24HR) using the Software for Intake Distribution Estimation Program (Iowa State University, 1996). This method provides a reliable

estimate of the usual dietary calcium intake of a group but not the usual intake of individuals (374). In the research setting, clarification of dietary calcium intake of individuals is crucial for interpreting and for comparing the role of dietary and supplement calcium on health outcomes over the longer term. Hence, valid dietary instruments for measuring dietary calcium intake of individuals are fundamental for advancing research in these areas.

Given the lack of a biologically valid biomarker of calcium intake, assessment of dietary calcium intake can be estimated by the use of dietary questionnaires, including repeated 24HR, food records, or food frequency questionnaires (FFQs) (375). No dietary assessment method is considered a "gold standard". However, the choice of the dietary assessment method is dependent on factors related to the study design, its objectives, target population, and resources (304). The FFQ is commonly used in nutritional epidemiology to rank or to compare nutrient intakes among individuals (304), and presents considerable advantages in terms of its practicality and cost-effectiveness (376). A calcium-focused FFQ would enable the inclusion of regular and specially fortified foods, resulting in an improvement in estimation of usual intakes of the nutrient. Furthermore, a calcium-focused FFQ requires less time to complete than a comprehensive FFQ, thus being less burdensome for the respondents and the researchers.

Many studies have evaluated the relative validity of FFQs to assess dietary calcium intake (377-383). However, FFQs may perform differently because of cultural, geographic, and demographic variations, as well as differences in food supply (384). Moreover, we could not identify a calcium-focused FFQ suitable for nutrition intervention studies that has been validated specifically in middle-aged and older Canadian women. The Canadian Multicentre Osteoporosis study (CaMOS) has developed a semiquantitative FFQ designed to estimate usual dietary calcium intake over the previous 12 months of Canadians between the ages of 16 and 24 years

and ≥25 years (311, 385). The relative validity of their dietary assessment tool for estimating dietary calcium intake has not yet been evaluated.

We hypothesized that a semiquantitative calcium-focused FFQ adapted from the CaMOS FFQ could be used to estimate usual dietary calcium intake in women ≥50 years old from Montreal, Canada. The modified FFQ was designed for the purpose of estimating dietary calcium intake of postmenopausal women in a randomized controlled trial that aims to assess the effects of supplemental calcium as compared with dietary calcium on vascular and bone health markers (ClinicalTrials.gov number, NCT01731340). This study's objectives are to test the hypothesis by evaluating the relative validity of the calcium-focused FFQ in comparison with four 24HR and the reproducibility in comparison with a second FFQ after a 1-month interval.

4.2 Methods and materials

4.2.1 Participants and study design

Community-dwelling women ≥50 years of age from the greater Montreal area (Quebec, Canada) were recruited via flyers and newspaper advertisements from April to September 2014. Women were excluded if they were unwilling to attend the baseline visit or planned to travel within the next month. Women were screened for eligibility through an initial telephone call and eligible participants were invited to attend a baseline visit at the study site. More specifically, participants attended 1 visit at the study site (Montreal General Hospital) and participated in 3 telephone interviews. Sociodemographic information and anthropometric measurements were also collected at baseline. Participants completed a first FFQ (FFQ1) at the initial visit in person. A second FFQ (FFQ2) was completed 1 month later by telephone with the participant at home. The participants also completed a 24HR at the initial visit, and then at 1 week, 2 weeks and 1 month over the telephone. All dietary assessments were completed with a registered dietitian

within 1 month ±1 week. We aimed to recruit 100 participants because this sample size falls within the reasonable range for validation studies to provide a power of 80% with a correlation coefficient in the range of 0.5 to 0.7 according to Willett (312).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the McGill University Health Centre Research Ethics Board. Written informed consent was obtained from all participants.

4.2.2 Development and administration of the FFQ

The questionnaire was adapted from the self-administered CaMOS semiquantitative FFQ, which was designed to estimate the dietary calcium intake of Canadians between the ages of 16 and 24 years and ≥25 years (80, 311). Given that self-administered FFQs may be challenging conceptually for participants and may potentially limit the usefulness of the data collected, we used an interviewer-administered calcium-focused FFQ to reduce the respondent burden and to increase the accuracy of the estimated dietary calcium intake. Following a literature review and with the collaboration of 2 nutrition researchers with expertise in nutrient intake assessments, the CaMOS FFQ was expanded to include new food items and food categories that consist of calcium-rich foods typically consumed by our target population (e.g., Greek yogurt, evaporated milk, condensed milk, mixed dishes made with cheese) as well as dietary sources of vitamin D that are available in Montreal, Quebec (386-388). The content validity of our 51-item FFQ was first assessed by 2 expert dietitians and then pilot tested with 4 female volunteers. Recommended changes were incorporated into the final version of the FFQ which was subsequently translated into French and back translated to English to ensure accuracy (**Supplemental Material**). We also randomly selected 20 additional female volunteers to assess the interrater agreement prior to

the first administration of the FFQ with participants of the main study to evaluate the relative validity of the tool. To assess the interrater agreement, 2 registered dietitians administered the FFQ to the 20 volunteers. These participants completed the FFQ twice, once at the initial encounter with 1 of the 2 dietitians and the second time with the other dietitian within a period of a week. During the completion of the FFQ, the volunteers were asked about the frequency (never, or times per month/week/day, as appropriate) and the usual portion size of consumption of the foods included in the FFQ during the previous month. Although food sources of vitamin D were included in the FFQ, the validity of the FFQ to assess dietary vitamin D intake is not included here.

Participants recruited for the FFQ validation component completed the FFQ with 1 of the 2 study dietitians and were asked about the usual frequency of consumption of the foods included in the FFQ during the previous month (e.g., "*How frequently did you drink milk during the past month?"*). The usual portion size consumed was asked separately for each item to enhance reporting accuracy (e.g., *"When you drank milk during the past month, how much did you usually drink each time?")*. An additional question was included at the end of the FFQ to capture calcium-rich foods that were not included in the questionnaire. For instance, the dietitian asked the participant whether there were foods that they had consumed at least once per week over the past month but not captured in the food list. The dietitian would verify the calcium content of the food per serving and then record it at the end of the FFQ if the food had greater than 50 mg of calcium per serving. Food models were used as visual aids to help participants estimate portion sizes during the first administration of the FFQ at the baseline visit.

4.2.3 Measurements

4.2.3.1 Anthropometry

Anthropometric measurements were performed in light clothing and without shoes at the baseline visit. Standing height was measured to the nearest 0.1 cm using a wall-mounted digital stadiometer (Seca 242; Seca, Hamburg, Germany) and weight was measured to the nearest 0.2 kg using a body composition analyser (Tanita TBF-310; Tanita Corporation, Arlington Heights, IL, USA).

4.2.3.2 Four 24HR as a reference method to evaluate the relative validity of the FFQ

The relative validity of the FFQ was evaluated against 4 nonconsecutive 24HR (24HRs) to capture the day-to-day variation in calcium intake while minimizing the burden imposed on the participants (375). All 24HRs were performed by a registered dietitian using the multiple-pass technique (329). Participants were asked to provide a complete list of foods they ate the day before the initial visit and the day before each scheduled telephone follow-up. Detailed instructions were provided to the participants during the initial visit to describe portion sizes, brand names, and recipes. A portion size guide with examples of visual cues using common household items was also provided as a handout to help participants estimate quantities and portion sizes during telephone interviews. This guide was adapted from a portion control guide available online (www.fns.usda.gov) (389).

4.2.3.3 Calculation of nutrient intakes

Intake data from each 24HR were entered into the Nutritionist Pro software version 5.2.0 (Axxya Systems, Stafford, TX, USA) to determine the nutrient content of food items using the Canadian Nutrient File 2010b database by Health Canada (373). Nutrient content of foods that were not available in the database was determined by verification of food labels, contact with

food manufacturers, or estimation from ingredient content by the study dietitian. The average calcium intake estimated by the 24HRs of each participant was calculated and used in the statistical analyses. An automated Microsoft (Redmond, WA, USA) Excel 2010 spreadsheet was created to tabulate the FFQ data. Averaged values of the calcium content of each item on the FFQ were obtained from the Canadian Nutrient File 2010b database and entered into the worksheet. Reported frequencies and quantities were entered, and the average daily dietary calcium intake for each item and the daily total were automatically calculated.

The contribution of dietary calcium from dairy and nondairy foods according to the FFQ2 was calculated. Dairy foods were further subdivided as fluid milk, cheese, and yogurt. Nondairy sources of calcium included in the analyses were plant-based beverages fortified with calcium and dark leafy green vegetables.

4.2.4 Statistical analyses

Normal distribution of the data was tested using the Shapiro-Wilk Test (390). Summary statistics were computed for baseline characteristics and presented as means and standard deviations (SDs) for normally distributed data, as medians and interquartile ranges (IQRs) for nonnormally distributed data, or as counts and percentages for frequencies. Interrater agreement was measured by calculating the intraclass correlation coefficient and 95% confidence intervals (95% CI) (391). In view of non-normally distributed intake data, Wilcoxon signed rank tests were used to compare the differences in calcium intakes between the FFQ1 and FFQ2, and between the FFQ2 and 24HRs. The Spearman rank correlation coefficient was calculated to measure the strength of the relationship between FFQ1 and FFQ2 for reproducibility (327). The nonparametric Spearman rank correlation was also calculated to measure the relationship between the FFQ2 and 24HRs. Although this does not provide an appropriate evaluation of

relative validity, the correlation coefficient is useful to compare with those reported in other validation studies (312).

To assess the relative validity of the FFQ to the four 24HR, calcium intakes derived from the FFQ2 were compared with the amount estimated by the 24HRs to cover the same period of time (i.e., the previous month). Calcium intakes estimated by the FFQ2 and 24HRs were first classified into quartiles to optimize the number of observations per category while being able to capture the small differences between groups (392). Cross-classification analysis was performed to determine the ability of the FFQ to rank calcium intakes by identifying the proportions of individuals correctly classified into the same, adjacent, or opposite quartiles (393). To test the relative agreement between FFQ2 and 24HRs adjusted for the amount of agreement that would be expected by chance, weighted Cohen's kappa (κw) defined with linear weights was calculated (393, 394). Values of $\kappa w > 0.80$ indicate very good agreement, between 0.61 and 0.80, good agreement; between 0.41 and 0.60, moderate agreement; between 0.21 and 0.40, fair agreement; and <0.20, poor agreement (395).

Bland-Altman plot analysis was performed and used as a graphical method to quantify the agreement of calcium intake as measured by the FFQ2 and the 24HRs (396). The means of the FFQ2 and 24HRs for each participant were plotted against the difference (FFQ2 – 24HRs) between each pair of observations. The mean difference and 95% limits of agreement (the mean difference ± 1.96 SD) were calculated to quantify the bias and the range of agreement between the 2 methods, respectively (397). To determine a clinically meaningful level of significance, a cutoff of 100 mg/d was applied for systematic bias, which is equivalent to approximately the amount of calcium that is usually found in a third of a standard serving of dairy food (383).

Sensitivity and specificity of the FFQ were calculated by first classifying dietary calcium intake as above or below the EAR for calcium for women >50 years of age based on estimated calcium intakes from the FFQ2 and 24HRs. *Sensitivity* was defined as the proportion of subjects with an intake below the EAR (i.e., <1000 mg/d) according to the 24HRs that also fell short on the FFQ. *Specificity* was defined as the proportion of participants with an intake above the EAR (i.e., ≥1000 mg/d) according to the 24HRs who also had estimated intakes above the EAR on the FFQ. The positive predictive value was calculated as the proportion of those who fell below the EAR on the FFQ whose 24HRs were <1000 mg/d. The negative predictive value was calculated as the proportion of those with an intake ≥1000 mg/d on the FFQ and 24HRs. A *P* value <0.05 was considered statistically significant. Statistical analysis was performed using the software R version 3.1.1 and the statistical software package IBM (Armonk, NY, USA) SPSS Statistics 22.0.

4.3 Results

In total, 115 women were enrolled in the study between April and December 2014. Six participants withdrew from the study because of loss of interest, and 1 participant did not complete the FFQ2 because of unavailability. Thus, 108 participants with complete FFQ and 24HRs data were included in the analysis. The flowchart depicting the selection process of participants is detailed in **Figure 4.1.** Participants had a mean age of 63.1 (SD 7.7) years and body mass index of 26.9 (SD 6.4) kg/m^2 (**Table 4.1**). Median daily dietary calcium intake estimated from the FFQ1 (895; IQR 619-1165 mg/d) was higher than the daily intake estimated from the FFQ2 (723; IQR 524-1033 mg/d) (*P*<0.001). The median daily dietary calcium intake estimated from the 24HRs (854; IQR 666-1068 mg/d) was also significantly greater than the daily intake estimated from the FFQ2 (*P*<0.001) (**Table 4.2**). The time required to complete the FFQ was typically less than 30 minutes.

4.3.1 Evaluation of the relative validity of the FFQ

The intraclass correlation coefficient to examine interrater agreement of the FFQ (n=20) between 2 interviewers was 0.93 (95% CI 0.82-0.97). In the study sample, the FFQ1 and FFQ2 had a Spearman rank correlation coefficient of 0.72 (P<0.001) and moderate agreement (κw=0.55). The Spearman rank correlation coefficient for FFQ2 and the 24HRs was 0.65 (P<0.001) and the 2 assessment methods had moderate agreement (κw=0.42).

Dietary calcium intakes were divided into quartiles to evaluate the ability of the FFQ to rank women into the same quartiles of intake as estimated by the four 24HR (**Table 4.3**). Cross-classification into quartiles showed that 85% of the women were classified into the same or adjacent quartiles, and 2.8% were classified into extreme quartiles (quartile 1: <619 mg/d; quartile 4: >1165 mg/d) of the FFQ and 24HRs, an indication of extreme misclassification of dietary calcium intake in the FFQ. The Bland-Altman plot demonstrated that the FFQ had a systematic bias of underestimating dietary calcium intake by approximately 99 mg/d (SD 295 mg/d) with 95% limits of agreement ranging from -677 to 480 mg/d (**Figure 4.2**).

The FFQ demonstrated 90% sensitivity for classifying participants with calcium intake below the EAR, and 57% specificity for classifying participants with intake above the EAR. Its positive predictive value for the proportion of participants with dietary calcium intake below the EAR was 80%. Its negative predictive value for the proportion of participants with dietary calcium intake above the EAR was 75%. The distributions of the estimated dietary calcium intakes according to the FFQ2 and the 24HRs are shown in **Figure 4.3**.

4.3.2 Calcium contribution from foods

The main food sources of calcium and their contribution to dietary calcium intake according to the FFQ2 are presented in **Table 4.4**. Dairy products provided a median contribution of 75% (95% CI 72-79%) of daily dietary calcium intake for women who reported consumption of milk, cheese, or yogurt. Of the 108 participants, 31% (*n*=33) reported consumption of plant-based beverages fortified with calcium which provided a median contribution of 15% (95% CI 6-23%) of dietary calcium intake in this subset of participants.

4.4 Discussion

We have developed an FFQ to estimate dietary calcium intake in women ≥50 years of age living in a major urban city in Canada. Our findings confirm the hypothesis that the FFQ may be used to estimate the usual dietary calcium intake of this specific population group. More precisely, the 51-item calcium-focused FFQ could identify relatively low from relatively high dietary calcium intakes in a cohort of women who were predominantly postmenopausal. Furthermore, we observed that the median usual dietary calcium intake estimated by the FFQ was below the EAR of 1000 mg/d from food sources alone, which is consistent with the population-based data (3).

This FFQ demonstrated good reproducibility as noted by the high correlation coefficient for the FFQ1 and FFQ2 that were administered 1 month apart. We observed a different median intake between the FFQs (lower estimated dietary calcium by the FFQ2), which could explain in part the moderate agreement. The difference in the estimated dietary calcium intake between the first and second administration of the FFQ may be attributed to various factors. First, participants might have overestimated the portion sizes or over reported the frequency of consumption during the completion of FFQ1 at the baseline visit as a result of response bias, such as social

desirability, by responding in a manner consistent with expected norms (310, 398). Second, increasing familiarity with the methods and portion sizes following their first administration of the FFQ could have reduced the self-report biases (399). The mode of administration on FFQ1 and FFQ2 could also have had a potential impact on the responses. However, results obtained from an in-person administration of the FFQ have been shown to be comparable to those obtained by telephone administration of the FFQ (400, 401). We cannot compare our observations with similar studies because reproducibility and repeatability are not commonly assessed in most FFQ validation studies (384). Nonetheless, we observed a correlation coefficient that is higher than that for repeat administrations of the FFQ in a time interval of 1 month or less (r=0.67) and between 1 to 6 months (r=0.70) (402).

Although the median estimates of dietary calcium intake from the FFQ2 and 24HRs were significantly different (723 *versus* 854 mg/d, P<0.001), dietary calcium intake derived from the FFQ2 was moderately correlated with intakes derived from the 24HRs (r_s=0.65, P<0.001). Our findings are similar to those in the literature, which report correlation coefficients in the range of 0.29 to 0.90 (377, 379, 382, 388, 403-409). Montomoli et al. reported the highest correlation coefficient (r=0.90) between a 15-item FFQ and 14 days of food record with 206 Italian women between the ages of 25 and 75 years (382). The use of food records in this study may have confounded their results because completing food records may lead to changes in food intake as the respondents become more aware of their diet (410). We used four 24HR as the reference method to minimize respondent burden and to reduce possible alteration in food intake secondary to anticipation of scheduled telephone calls. Nonetheless, our FFQ had a higher correlation coefficient (r_s=0.65) with the 24HR method than other FFQ validation studies in postmenopausal women of other ethnic backgrounds. The reported correlation coefficients in

studies that assessed the relative validity of the FFQ against the 24HR method ranged from 0.29 to 0.64 (379, 405, 411).

Very few studies in women examined the agreement between the FFQ and a reference method using κ statistics. The range for κw reported in the literature is between 0.20 and 0.75 (379, 404, 407). In our study, agreement between the FFQ2 and 24HRs was moderate (κw=0.42), which was in line with another study that was conducted in older women. Satalic et al. evaluated the validity of their FFQ against a single multiple-pass 24HR in 333 Croatian postmenopausal women and reported a κw of 0.43 (379). In our study, the agreement between the FFQ and 24HRs as demonstrated by the Bland-Altman plot is stronger than other studies that have evaluated the relative validity of calcium-focused FFQs in postmenopausal women (systematic bias range: 121-221 mg) (379, 388, 405). Although our results showed that the systematic bias was more evident for higher intake levels, this was expected as variability increases with higher levels of calcium intake. Agreement between the FFQ and 24HRs can be improved by comparing the average FFQ estimates (FFQ1 and FFQ2) in lieu of using only FFQ2 to reduce the effect from interindividual variation over time. We chose not to use the average estimates because the data collected from FFQ1 did not reflect the same time frame as the four 24HR and FFQ2. Still, our FFQ underestimates dietary calcium intake by a relatively small amount that is <100 mg/d, which is equivalent to approximately one-third of a serving of milk or milk alternatives, or 10% of the EAR. This level of underestimation by the FFQ likely misclassified some individuals into adjacent quartiles in the cross-classification analysis. Although there were some misclassifications, only 2.8% of the participants were classified into the opposite extreme quartiles by the FFQ. Because of its semiquantitative nature and high sensitivity, our FFQ is a useful dietary assessment tool to rank dietary calcium intakes in epidemiological studies.

Milk, cheese, and yogurt accounted for a calcium intake that was approximately half of the EAR (median=541 mg/d) and contributed to 75% of the dietary calcium intake of participants who reported consuming dairy products. Of the 108 women in our study, only 1 participant reported not to have consumed milk or dairy products. Although similar data from Canada are not currently available, the contribution of calcium from dairy foods that we observed in our study is very similar to what was reported in the 2009-2010 US National Health and Nutrition Examination Survey (412). Consumption of alternative beverages to milk, such as soy and almond beverages fortified with calcium and vitamin D, was reported by approximately one-third of the participants and only contributed the equivalent of 15% of the EAR among those who consumed these products. Although these milk alternative beverages are not the primary source of dietary calcium among women in our cohort, the inclusion of both dairy and nondairy sources of calcium-rich food items in the FFQ is important to capture a better estimate of total dietary calcium intake and to accurately rank intakes in epidemiological studies.

The present study has several strengths. First, our FFQ is composed of food items with relatively important dietary calcium contribution in postmenopausal women from Montreal, Canada, according to a recently published study in this specific population (386). In addition to cultural and geographic variations, FFQ items must also be adapted to include trending calcium-rich foods to accurately reflect dietary calcium in this specific population. Second, in addition to the use of correlation coefficients, our study included different and relevant statistical analyses to evaluate the relative validity of the FFQ including κ statistics, Bland-Altman analysis, and the sensitivity and specificity analyses (383, 396). Finally, the design of our study allowed us to evaluate the relative validity, reproducibility and sensitivity of the FFQ in identifying women with dietary intakes below the EAR for calcium, which are critical characteristics of a valid FFQ

(413). The quantitative information on the relative validity of the 51-item calcium-focused FFQ substantially enhances the interpretation of any study investigating the effect of dietary calcium interventions on health outcomes (312). This study also has limitations. The sample was predominantly composed of postmenopausal white women with a high level of education. Although the participants were recruited from one geographic region, a large variety of multicultural foods including tofu, Italian dishes made with cheese, Greek style yogurt, and nondairy sources of dietary calcium, are easily accessible in the major urban city. We have included these foods in our FFQ because they have been observed to be commonly consumed items by postmenopausal women in Montreal, Canada (386). Volunteer bias may have also been present, thus potentially limiting the generalizability of the findings to the general Canadian women (310). Our findings may not be applicable to populations that abstain from consumption of dairy foods because 99% of the study participants consumed dairy foods. However, our FFQ included nondairy sources of calcium-rich foods to capture the contribution of dietary calcium intake from nondairy alternatives in individuals who avoid dairy. Of all the nondairy calcium sources included in our FFQ, fortified plant-based beverages contributed the most dietary calcium (15%) in participants who reported consumption of these beverages. There are also potential limitations related to the dietary intake recorded by the 24-hour dietary recall instrument. Because both the FFQ and 24HR methods rely on memory and perception of serving sizes, weighed food records may be a better reference method to improve the accuracy of the estimated intake (312). We chose the repeated 24HR method to reduce respondent burden and to maximize participant retention. To minimize the distortion of portion sizes using the 24HR method, we provided portion size tools to participants at the initial visit with the dietitian to help participants properly estimate portions sizes. We also collected 4 nonconsecutive days of dietary

recall. Although they were completed on weekdays only, Nelson and Bingham (414) demonstrated that a 3- to 4-day 24-hour recall randomized to weekday variations provided minimal differences in nutrient intake from seven 24 hour dietary recalls.

In summary, we accept the hypothesis that the 51-item semiquantitative FFQ may be used to assess usual dietary calcium intake in this population. The FFQ is a useful tool to discriminate low (<600 mg/d) and high (>1000 mg/d) dietary calcium intake in postmenopausal women and to rank dietary calcium intake for epidemiological studies. To ensure the generalizability of our findings, the FFQ should be evaluated in a larger and more diverse population of women ≥50 years old across Canada.

Table 4.1 Characteristics of participants

Characteristics	Means ± SD
Age (y)	63.1 ± 7.7
Postmenopausal (*n*, %)	106 (98.1)
White (*n*, %)	97 (89.8)
BMI (kg/m^2)	26.9 ± 6.4
Retired (*n*, %)	62 (57.4)
Level of education (*n*, %)	
High school or less	38 (35.2)
Pre-university	26 (24.1)
University	44 (40.7)
Smoking history (*n*, %)	
Smoker	5 (4.6)
Ex-smoker	46 (42.6)
Never	57 (52.8)
Alcohol consumption (*n*, %)	
>9 drinks per week	9 (8.3)
1-9 drinks per week	77 (71.3)
None	22 (20.4)
Season of first visit (*n*, %)[a]	
Summer	78 (72.2)
Winter	30 (27.8)

n=108. Data are means and standard deviations.
BMI, body mass index; SD, standard deviation.
[a] Summer season (May to October); Winter (November to April)

Table 4.2 Average daily energy intake estimated by the 24HRs and dietary calcium intakes estimated by the FFQ and 24HRs

Characteristics	Medians	IQRs
Energy intake (kJ/d)[a]	7368	6200-8429
Dietary calcium intake (mg/d)		
FFQ1[b]	895*	619-1165
FFQ2[c]	723	524-1033
24HRs	854*	666-1068

Data are presented as medians and IQRs .
24HRs, 4 nonconsecutive 24-hour recalls; FFQ, food frequency questionnaire; IQR, interquartile range.
[a] Energy intake estimated by the 24HRs.
[b] FFQ1 reflects usual dietary calcium intake of the previous month before the baseline visit.
[c] FFQ2 reflects usual dietary calcium intake of the previous month before the final follow-up.
* Estimated dietary calcium intake significantly higher than that estimated from FFQ2, $P<0.001$.

Table 4.3 Cross-classification (%) of dietary calcium intake into quartiles by the FFQ2 and 24HRs with weighted κ statistics

Method of dietary intake assessment	FFQ							
	Q1	Q2	Q3	Q4	Same quartile	Same ± adjacent quartile	Opposite quartile	κw
24HRs	59.2	33.3	33.3	55.6	45.4	85.2	2.8	0.42*

24HRs, four nonconsecutive 24-hour recalls; FFQ, food frequency questionnaire; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4.
* Weighted kappa (κw) statistic $P<0.001$ indicates moderate agreement.

Table 4.4 Main food sources of calcium as assessed by the FFQ2 by categories of calcium-rich foods

Food	No. of consumers	% of participants	Intake (mg/d)[b]		Contribution (%)[b]	
			Medians	95% CI	Medians	95% CI
Dairy	107	99	541	463-591	75	72-79
Fluid milk	99	92	218	160-273	29	23-37
Cheese	103	95	105	83-151	17	14-20
Yogurt	94	87	100	75-128	13	9-16
Fortified plant-based beverages[a]	33	31	136	48-169	15	6-23
Dark green leafy vegetables	103	95	19	14-23	3	2-4

n=108
FFQ2, food frequency questionnaire completed at 1 month.
[a] Plant-based beverages fortified with calcium and vitamin D. Soy beverages not fortified with calcium are not included.
[b] Intake and contribution of dietary calcium were calculated among participants who reported consumption of the food item in the past month.

Figure 4.1 Flowchart of participants.

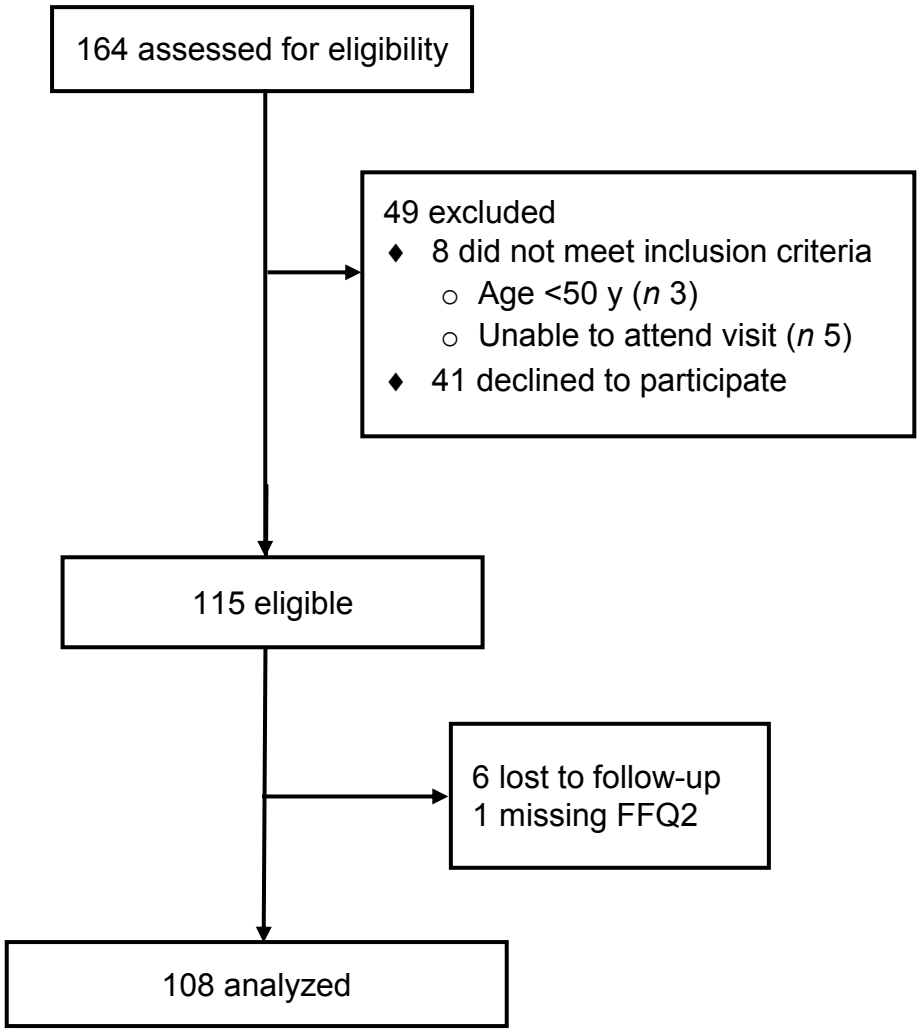
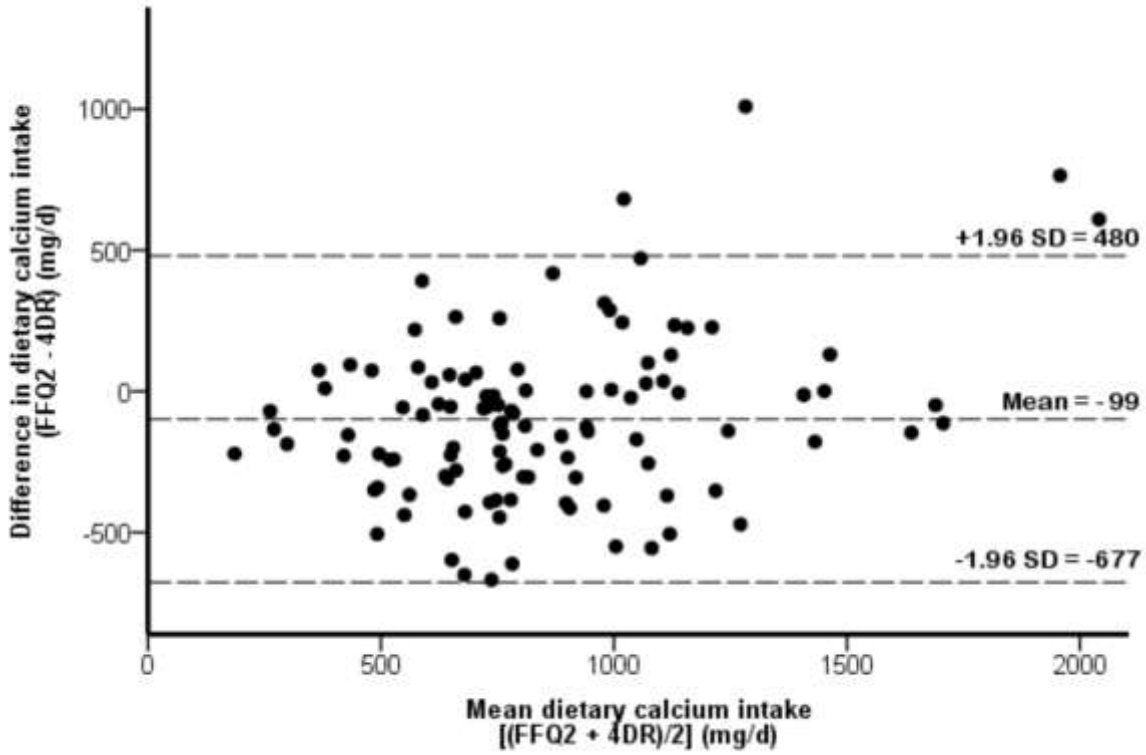


Figure 4.2 Bland-Altman plot assessing the agreement between the FFQ2 and the 24HRs for estimating dietary calcium intake in women ≥50 years of age.



FFQ2 indicates food frequency questionnaire completed at 1 month; 24HRs, 4 nonconsecutive 24-hour recalls. Difference in dietary calcium intake = FFQ2 – 24HRs.
Mean dietary calcium intake = (FFQ2 + 24HRs)/2. SD = 295 mg/d.

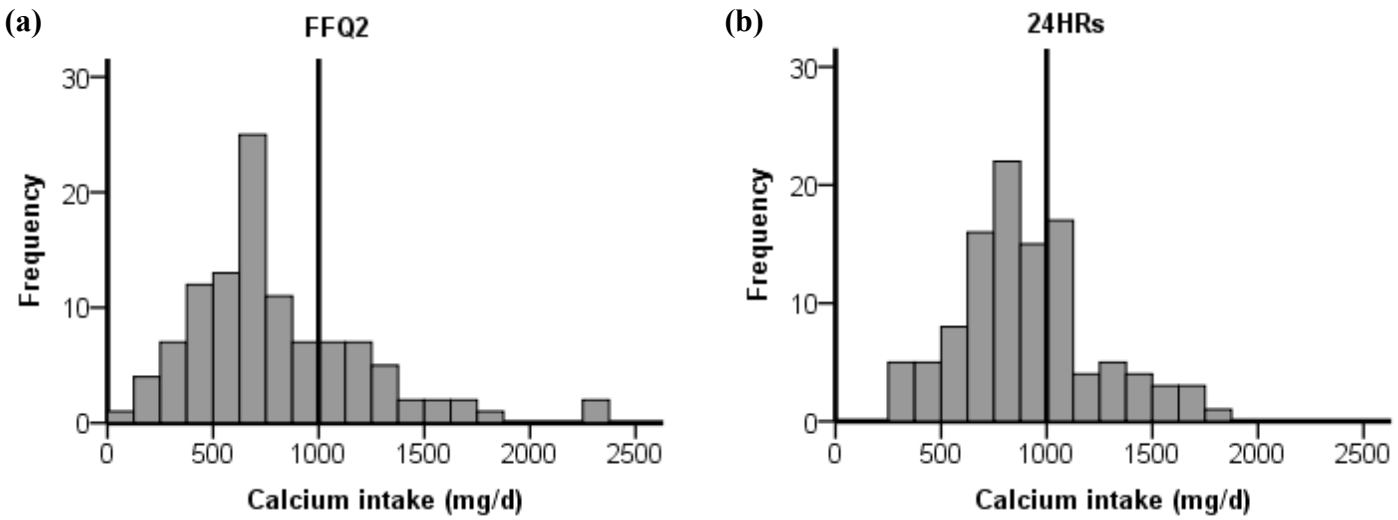


Figure 4.3 Distribution of dietary calcium intake assessed by the FFQ2 (A) and the 24HRs (B) in comparison to the EAR for women >50 years of age.
FFQ2 indicates food frequency questionnaire completed at 1 month; 24HRs, 4 nonconsecutive 24-hour recalls; EAR, Estimated Average Requirement. Solid line represents the EAR (1000 mg/d) for women >50 years old.

Supplemental Material. The 51-item food frequency questionnaire

How often (on average) have you eaten the following items during the *past month*?

Food item	Never	Servings per month	Servings per week	Servings per day	Serving size
Milk **fortified with calcium** to drink	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Milk **fortified with calcium** in cereal	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Milk to drink	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Milk in cereal	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Milk/Cream in tea/coffee	□	_____	_____	_____	□ 15 ml (1 tbsp) □ 30 ml (2 tbsp) □ 60 ml (4 tbsp)
Alternative milk to drink – **fortified with calcium** *(□ soy, □ almond, □ rice, □ hemp, □ oat)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Alternative milk in cereal – **fortified with calcium** *(□ soy, □ almond, □ rice, □ hemp, □ oat)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Alternative milk in tea/coffee – **fortified with calcium** *(□ soy, □ almond, □ rice, □ hemp, □ oat)*	□	_____	_____	_____	□ 15 ml (1 tbsp) □ 30 ml (2 tbsp) □ 60 ml (4 tbsp)
Soy beverage to drink – <u>not</u> fortified with calcium	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Soy beverage in cereal – <u>not</u> fortified with calcium	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Evaporated milk	□	_____	_____	_____	□ 15 ml (1 tbsp) □ 45 ml (3 tbsp) □ 125 ml (0.5 cup)
Sweetened condensed milk	□	_____	_____	_____	□ 15 ml (1 tbsp) □ 30 ml (2 tbsp) □ 60 ml (4 tbsp)
Milk desserts – homemade *(ex: tapioca, rice pudding)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup)
Milk desserts – prepared/pre-packaged *(ex: tapioca, rice pudding)* *(1 small container = 113 g)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 1 container (small)

Food item	Never	Servings per month	Servings per week	Servings per day	Serving size
Milk desserts – homemade (**with fortified alternative milk**)	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup)
Milk desserts – prepared/pre-packaged (with fortified alternative milk) *(ex: tapioca, rice pudding)* *(1 small container = 112 g)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 1 container (small)
Cream soups prepared with milk	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 160 ml (2/3 cup) □ 250 ml (1 cup)
Cream soups prepared with alternative milk – **fortified with calcium**	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 160 ml (2/3 cup) □ 250 ml (1 cup)
Ice cream, ice milk or frozen yogurt	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 250 ml (1 cup) □ 375 ml (1.5 cups)
Greek yogurt *(plain or flavored)* *(1 small container = 100 g)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 200 ml (0.75 cup) □ 250 ml (1 cup) □ 1 container (small)
Yogurt to eat or drink - regular *(plain or fruit flavored)* *(1 small container = 100 g)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 200 ml (0.75 cup) □ 250 ml (1 cup) □ 1 container (small)
Cottage cheese *(1 small container = 113 g)*	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 175 ml (0.75 cup) □ 250 ml (1 cup) □ 1 container (small)
Fresh, soft or cream cheese *(brie, camembert, goat, ricotta, feta)*	□	_____	_____	_____	□ 14 g (0.5 oz) □ 28 g (1.0 oz) □ 56 g (2.0 oz)
Firm or processed cheese *(including in sandwich or mixed dish)* *(blue, fontina, cheddar, Swiss, gouda, colby, edam, provolone, brick)*	□	_____	_____	_____	□ 14 g (0.5 oz) □ 28 g (1.0 oz) □ 56 g (2.0 oz)
Hard cheese *(gruyère, romano, parmesan)*	□	_____	_____	_____	□ 15 ml (1 tbsp) □ 30 ml (2 tbsp) □ 45 ml (3 tbsp)
Pizza *(medium 12", 1/8 = 1 slice, approx. 100 g)*	□	_____	_____	_____	□ 1 slice □ 2 slices □ 3 slices

Food item	Never	Servings per month	Servings per week	Servings per Day	Serving size
Pasta with cream or cheese sauce	□	_____	_____	_____	□ 250 ml (1 cup) □ 375 ml (1.5 cups) □ 500 ml (2 cups)
Lasagna *(1 piece = 7.5 cm x 9 cm)*	□	_____	_____	_____	□ 0.5 piece □ 1 piece □ 1.5 piece
Pasta stuffed with cheese *(ex. tortellini, ravioli)*	□	_____	_____	_____	□ 250 ml (1 cup) □ 375 ml (1.5 cups) □ 500 ml (2 cups)
Oranges *(1 fruit = 1 medium sized fruit)*	□	_____	_____	_____	□ 0.5 fruit □ 1 fruit □ 2 fruits
Orange juice – **fortified with calcium**	□	_____	_____	_____	□ 125 ml (0.5 cup) □ 160 ml (2/3 cup) □ 250 ml (1 cup)
Canned salmon or sardines **with bones**	□	_____	_____	_____	□ 28 g (1 oz) □ 56 g (2 oz) □ 84 g (3 oz)
Salmon – canned or fresh without bones	□	_____	_____	_____	□ 56 g (2 oz) □ 84 g (3 oz) □ 112 g (4 oz)
Other fish	□	_____	_____	_____	□ 56 g (2 oz) □ 84 g (3 oz) □ 112 g (4 oz)
Broccoli – cooked or raw	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Dark leafy greens – cooked *(bok choy, kale, gailan (chinese broccoli), collards, dandelion or beet greens, spinach)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Dark leafy greens – raw *(bok choy, kale, gailan (chinese broccoli), collards, dandelion or beet greens, spinach)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Dried (or canned) beans or peas *(navy, pinto, kidney, chick peas, lentil, etc.)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
White bread, buns, rolls, bagels, pita, tortilla	□	_____	_____	_____	1 serving = 1 slice ½ bagel ½ pita
Whole wheat bread, buns, rolls, bagels, pita, tortilla	□	_____	_____	_____	1 serving = 1 slice ½ bagel ½ pita

Food item	Servings per				Serving size
	Never	month	week	day	
Pancakes, waffles *(1 small piece = 10.2 cm diameter)*	□	_____	_____	_____	□ 1 piece □ 2 pieces □ 3 pieces
Tofu, firm *(prepared with calcium sulfate)*	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Tofu, silken	□	_____	_____	_____	□ 60 ml (0.25 cup) □ 125 ml (0.5 cup) □ 250 ml (1 cup)
Almonds	□	_____	_____	_____	□ 30 ml (2 Tbsp) □ 60 ml (0.25 cup) □ 125 ml (0.5 cup)
Margarine	□	_____	_____	_____	□ 5 ml (1 tsp) □ 15 ml (1 tbsp) □ 45 ml (3 tbsp)
Egg, large (with yolk)	□	_____	_____	_____	□ 1 egg □ 2 eggs □ 3 eggs
Liver *or liver pâté*	□	_____	_____	_____	□ 56 g (2 oz) □ l12 g (4 oz) □ 168 g (6 oz)
Deli meat *(salami, bologna, luncheon meat)* *(1 slice = 1 oz)*	□	_____	_____	_____	□ 1 slice □ 2 slices □ 3 slices
Meat *(pork, poultry, beef, sausage, bacon)*	□	_____	_____	_____	□ 56 g (2 oz) □ l12 g (4 oz) □ 168 g (6 oz)
Energy bars *(ex: Cliff, Luna bars, SlimFast, PowerBar)* *(small bar = 48g, large bar = 60 g)*	□	_____	_____	_____	□ 0.8 bars (small) □ 1 bar (large)
Meal replacement drink *(ex: Ensure, Boost, etc.)* *(1 bottle = 235 ml)*	□	_____	_____	_____	□ 0.5 bottle □ 1 bottle □ 1.5 bottle

Are there any other foods not mentioned above that you usually eat <u>at least once per week</u>?

Other foods that you usually eat at least once per week	Usual serving size	Servings per week
(a)		
(b)		
(c)		
(d)		
(e)		

Bridge Statement 3

The results of the FFQ validation study in Chapter 4 demonstrated that the 51-item calcium-focused FFQ was a suitable tool to discriminate low intakes (<600 mg) from high intakes (>1000 mg/d) of dietary calcium in postmenopausal women living in Montreal. This tool can be used in epidemiological studies investigating the effect of dietary calcium on health outcomes to estimate usual dietary calcium intake in postmenopausal women.

Consistent with other studies and national survey data, findings from the validation study in Chapter 4 showed that milk and milk products are the highest contributors of dietary calcium intake, providing a median calcium intake of 541 mg/day (95% CI 463-591). When broken down into individual dairy products, milk, cheese and yogurts contributed 29%, 17% and 13% of total calcium intake, respectively. Conventional dietary guidelines recommend milk and milk products altogether, but emerging evidence suggests that FMPs, which include cheese and yogurt, and non-FMPs may have varying effects on body composition, CVD and diabetes (46). More precisely, FMPs may be associated with reduced CVD risk, whereas the association between milk intake and risk of all-cause and cardiovascular mortality have been supported by mixed findings (41, 47, 84, 88). It has been hypothesized that FMPs may exert more benefits on bone health than non-FMPs. Findings from a 2017 meta-analysis reported an association between higher intakes of yogurt and cheese and reduced risk of hip fractures in both men and women combined (415). However, given the sex difference in risk of fractures and osteoporosis, further clarification of the relationship between dairy foods, categorized as fermented and non-fermented, and bone health markers in postmenopausal women is needed. Chapter 5 is a systematic review and meta-analysis designed to examine the association between FMPs and bone health indicators in postmenopausal women.

CHAPTER 5

Manuscript 3: Systematic Review and Meta-Analysis

Published: *Advances in Nutrition*, 2020;11(2):251-65

Fermented milk products and bone health in postmenopausal women: a systematic review of randomized controlled trials, prospective cohorts and case-control studies

Angel M. Ong[1,2], Kai Kang[1, 3], Hope A. Weiler[1], Suzanne N. Morin[2,4]

Authors' Affiliation:

[1] School of Human Nutrition, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

[2] Research Institute of the McGill University Health Centre, 5252 de Maisonneuve Ouest, Montreal, Quebec, Canada, H4A 3S5

[3] Department of Research and Surveillance Evaluation, Shanghai Center for Health Promotion, 358 Jiaozhou Road, Jingan Area, Shanghai, China, 200040

[4] Department of Medicine, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada, H3G 1Y6

Abstract

Milk and milk product consumption is positively associated with bone mineral density (BMD). Emerging evidence suggests that fermented milk products (FMPs) may have specific benefits on skeletal health. We conducted a systematic review and meta-analysis to assess the effect of FMPs on bone health indicators in postmenopausal women given their increased risk for osteoporosis and fragility fractures. Electronic databases were searched for randomized controlled trials (RCTs), prospective cohort and case-control studies that examined the relation between FMPs and bone health outcomes (fracture incidence, BMD, BMD T-score, percent change in bone turnover markers) in postmenopausal women. Two reviewers independently conducted abstract and full-text screenings and data extractions. Risk of bias was assessed using the RoB 2.0 tool and the Newcastle-Ottawa scale for interventional and observational studies. Pooled relative risks (RR) were obtained using a random-effects model by the Dersimonian-Laird method. Three RCTs, 3 prospective cohorts and 3 case-control studies met the inclusion criteria. Results of the meta-analysis of 3 cohort studies (n=102,819) suggest that higher yogurt consumption was associated with reduced hip fracture risk (pooled RR: 0.76; 95% CI: 0.63, 0.92, I^2=29%), but no difference in hip fracture risk was found between higher and lower cheese consumption (pooled RR: 0.89; 95% CI: 0.73, 1.10, I^2=0%). Case-control studies revealed that cheese intake had either a null or protective effect against osteoporosis (BMD T-score ≤-2.5). Daily yogurt or cheese intervention (<2 months) decreased bone resorption marker concentrations, but had no effect on bone formation markers. In postmenopausal women, of the FMPs studied, only greater yogurt consumption was associated with a reduced risk of hip fracture compared to low or no intake. Daily cheese intake may be associated with higher BMD T-scores, but evidence was limited. Additional and longer-term trials examining these relationships are warranted.

5.1 Introduction

Osteoporosis and osteoporotic fractures are public health concerns, especially in older women. This skeletal disease, characterized by low bone mass and micro-architectural deterioration of bone tissue, affects approximately 200 million women globally (56, 416, 417). Although age-related bone loss affects both men and women, the decline of bone mass is accelerated at menopause when bone resorption exceeds bone formation (418). Moreover, fracture rates are higher in elderly women than in men, an increased risk that is not only a result of predisposed genetic differences between the sexes, but is also attributable to other factors affecting the preservation of bone mass in later adulthood.

For instance, reaching maximal peak bone mass by early adulthood, physical activity, and adequate nutrition are major factors that affect the retention of bone mass across the lifespan. Amongst the bone-building nutrients, calcium, an important component of bone, plays a primary role in osteoporosis prevention as a modifiable factor that helps reduce bone loss. Findings from a systematic review on dietary calcium intake among adults showed that women generally have a lower average calcium intake than men (419). Furthermore, national surveys from North America indicated that over 80% of women $\geq$ 50 years have a dietary calcium intake that falls below the current Recommended Daily Allowance of 1,200 mg/day (3, 4, 58). To reduce the global burden associated with osteoporosis, optimizing calcium intake is necessary. Although calcium can be found in many foods, milk and milk products such as yogurt and cheese are valued as good or excellent sources of calcium (62, 420).

Evidence from observational studies and randomized controlled trials (RCTs) has shown a positive association between milk or total milk product intake and bone mineral density (BMD) (421-423). Reduced fracture risk is the key clinical outcome sought in bone health interventions,

and yet the impact of milk and milk product consumption on fracture risk, including risk of hip fracture, remains unclear (424-426). Milk, yogurt and cheese have similar yet distinct nutrient profiles that vary in part due to the fermentation process. Fermented milk and fermented milk products (FMPs), also known as cultured milk products, are milk products prepared by lactic acid fermentation (427). The bacterial cultures in cheese are less active than those found in some yogurts in which live bacteria remain active postconsumption. Results from a large cohort study reported an inverse association between FMP consumption and the fracture risk in middle-aged and older women and, in contrast, high milk intake was associated with greater fracture risk (40). Michaëlsson et al. (40) proposed that the higher content of D-galactose found in milk compared to FMPs may act as a prooxidant and promote inflammation based on the positive association between milk intake and markers of oxidative stress (urine 8-iso-PGF2α) and inflammation (IL-6), whereas the probiotic content of FMPs may exhibit antioxidant and anti-inflammatory properties that can benefit bone health. Urine 8-iso-PGF2α and serum IL-6 have previously been shown to be negatively associated with BMD and stimulate bone resorption, respectively (428, 429). Moreover, emerging evidence suggest favorable effects of probiotic supplementation on bone health and reduction in proinflammatory cytokines such as TNF-α and IL-1β (300, 301). Although robust data in humans demonstrating these effects are lacking, these findings are of possible public health interest given that dietary guidelines often recommend milk and milk products collectively yet probiotics are only found in FMPs.

Recently, Bian et al. (415) conducted a meta-analysis to examine the association of different types of milk products on hip fracture risk in men and women and found that yogurt and cheese consumption, but not milk consumption, was associated with reduced fracture risk. However, whether the observed associations differed between men and women was not explored

in their study. In view of the higher prevalence in osteoporosis and greater fracture incidence in postmenopausal women than in older men (416, 417), it is important to examine the relation between FMP intake and various bone health indicators in postmenopausal women specifically. The purpose of this systematic review is to summarize the evidence on the association of FMP consumption on skeletal outcomes and bone health indicators in postmenopausal women.

5.2 Methods

This review was registered on the International Prospective Register of Systematic Reviews in 2018 (PROSPERO) as CRD42018085232, and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (430).

Literature search

A literature search in Embase Classic+Embase (1947-present, OvidSP), MEDLINE (1946-present, OvidSP), PubMed (1946-present, PubMed), CINAHL Plus (1937-present, EBSCOhost) and the Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library) was conducted up to January 9 2019 for studies of FMP consumption and bone health indicators. An expert librarian was consulted to generate a list of keywords and MeSH terms to conduct the search (**Supplemental Methods**). Searches were not limited by year of publication and no language restrictions were applied. The reference list of all included studies and nutrition research journals were hand searched individually to identify additional studies eligible for this systematic review. Abstracts of conference proceedings and gray literature were excluded.

Study selection

We included RCTs, prospective cohort studies, and case-control studies that examined the relation between FMP consumption and a bone health outcome in postmenopausal women or women ≥55 years old. Studies evaluating the consumption of FMPs were considered in this

systematic review regardless of the type, frequency or dose of FMP, or the method of assessment of FMP intake. All studies that compared the consumption of FMPs to that of non-FMPs, low consumption, no consumption or placebo were included. Studies that included a combination of milk product intake were included only if it was possible to quantify the intake of fermented and non-FMP intake. Studies with mixed interventions were excluded.

Studies were included in this systematic review if they reported on 1 of the following outcomes: *1*) incidence of vertebral or non-vertebral fractures; *2*) percent change from baseline in BMD of the lumbar spine, the total hip, or the femoral neck. Studies that reported a percentage change in bone mineral content of any site, BMD T-score of the lumbar spine, total hip, or femoral neck, and bone turnover markers were also included in this review. The relative percentage change in BMD following the intervention was a primary outcome of interest (431-435). Although forearm BMD has been suggested as an alternative when BMD of central sites cannot be measured, the BMD of this peripheral site was not included. as our scoping search yielded no prospective data. Currently, dual-energy x-ray absorptiometry (DXA) is the gold standard assessment for measuring BMD and predicting fracture risk in the clinical setting. BMD can also be classified and expressed as a T-score, which is the difference between a patient's BMD and that of a young adult reference population expressed in standard deviation (SD) scores from the reference (436). Individuals with T-scores of $\leq$-2.5 meet the World Health Organization's criterion for diagnosing osteoporosis (437, 438). We included bone turnover markers as secondary outcomes of interest because these predict the rate of bone loss as well as the risk of fragility fractures, independently of BMD (439-444). Changes in bone turnover markers also occur rapidly in response to osteoporosis treatments and are associated with fracture reduction (445). Studies that reported a change in bone formation markers [osteocalcin,

bone-specific alkaline phosphatase (BSAP), procollagen type 1 N-terminal propeptide (P1NP), procollagen type 1 C-terminal propeptide] or bone resorption markers [tartrate-resistant acid phosphatase 5b (TRACP 5b), pyridinoline, deoxypyridinoline, C-terminal telopeptide of type 1 collagen (CTX), N-terminal telopeptide of type 1 collagen (NTX)] were included.

Data extraction

Study selection, data extraction and quality assessment were performed independently by two reviewers (AMO and KK) and disagreement was resolved by consensus or in consultation with a third reviewer. The following information was extracted for each study: name of first author, year of publication, country or region where the study was performed, study design, duration of the study, sample size, recruitment and study completion rates, participant characteristics (age, ethnicity, level of education, smoking status, osteoporosis status, medication use, dietary intake of FMPs and non-FMPs, calcium and/or vitamin D supplementation, physical activity level), effect estimates of outcome measurements, and variables adjusted for in the multivariate models of each study. In observational studies, only the lowest and highest levels of intake were extracted. Authors were contacted to obtain information on missing or unreported data. When no response was received from the author, then the study was excluded from this review.

Risk of bias and quality assessment

Risk of bias in RCTs was assessed using the Cochrane Risk of Bias Tool 2.0 (RoB 2.0) (446). Each component was categorized as “low risk”, “some concerns”, or “high risk”. Methodological quality of observational studies was assessed using the Newcastle-Ottawa Scale (447). High-quality items were awarded 1 star, and the highest quality studies were awarded up to 9 stars. Studies with 0–3, 4–6, and 7–9 stars were considered as low, moderate, and high

quality, respectively. We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess the quality of evidence across studies for each outcome (448).

Data synthesis and analysis

Relative risks (RRs) from each study were combined using a random-effects model based on the Dersimonian-Laird method in pooling estimates to minimize problems of heterogeneity (449). Heterogeneity was evaluated using both Cochran's *Q* test and I^2 statistics. The I^2 statistical test was performed to complement the Cochran's *Q* test given that the latter test has low power to detect true heterogeneity when the number of studies is small, while the former test does not depend on the number of studies (450, 451). A significant Q value (*P*-value <0.05) or an I^2 value >50% was considered a considerable level of heterogeneity. When considerable heterogeneity was observed, reasons for heterogeneity were explored in subgroup analyses. Meta-regression analysis can be considered if the number of studies included exceeds 5 to explore the sources of variability. Due to the limited number of studies, meta-regression analysis was not performed. Meta-analyses were performed using the 'metafor' package of the R software (http://r-project.org/, version 3.1.1).

5.3 Results

Search results

The PRISMA flow diagram illustrating the flow of articles through the search and selection process is shown in **Figure 5.1**. The initial search yielded 1028 articles, and after removing 510 duplicates, 518 articles were identified for title and abstract screening. The selection process yielded 37 potentially relevant full-text publications, of which we contacted 7 authors to obtain missing information and 4 responded (452-455). One study with a missing

effect estimate for 1 of their subgroups of participants was included in our narrative review (456). Two studies were excluded due to no response (457) and unsuccessful contact with the corresponding authors (458). Following full-text review, we identified 9 studies of FMP intake in postmenopausal women that reported on hip fractures (*n*=4), BMD T-scores (*n*=2), and bone turnover markers (*n*=3). There was no study with vertebral fracture as an outcome.

Hip Fractures

Yogurt consumption

No RCTs examined hip fracture, or any type of fragility fractures, as an outcome. Evidence from 3 prospective cohort studies indicate that the highest level of yogurt consumption compared to the lowest intake category was associated with a reduced risk of hip fracture (**Table 5.1**). Data on hip fractures in women ≥55 years old from the Framingham Original Cohort (453) and the Swedish Mammography Cohort (452) were obtained from the authors to meet the inclusion criteria of this review. A total of 469 women (mean age 77±5 years) from the Framingham Original Cohort were included in this review, with 76 women sustaining an incident hip fracture during a mean follow-up of 11.6 (range 0.04-21.9) years. Intake was assessed at baseline with a semi-quantitative food frequency questionnaire. The association of any yogurt intake (>0 serving/week; 1 serving = 240 mL) on hip fracture risk as compared to no yogurt intake was not significant (RR: 1.12; 95% CI: 0.66-1.95) (453). In the Nurses' Health Study, 80,600 postmenopausal women (mean age 54 [range 34-60] years) were followed for a mean duration of 20.8 years, during which a semi-quantitative food frequency questionnaire was administered 9 times during the follow-up period. No significant association between yogurt consumption and hip fracture risk in postmenopausal women was observed (459). Michaëlsson et al. (452) found a 29% reduced risk of hip fracture in the subcohort of 21,750 Swedish women

(mean age 63±5 years) who reported a higher consumption of FMP (≥2 servings/day of yogurt and soured milk; 1 serving = 200 mL) than those who were nonconsumers at baseline (RR: 0.71; 95% CI: 0.63-0.79). The meta-analysis of 3 prospective cohort studies resulted in a RR of incident hip fractures of 0.76 (95% CI: 0.63-0.92; *P*-heterogeneity = 0.25, I^2 =29%) (**Figure 5.2**).

Cheese consumption

Two prospective cohort studies and 1 case-control study examining the association between cheese intake and hip fractures were identified (**Table 5.2**). Cheese intake was not associated with hip fracture risk in postmenopausal women. In the case-control study, which included 241 cases of women [median age 64 (range 45-74] years) hospitalized for a hip fracture and 719 controls, there was no association (odds ratio [OR]: 1.0; 95% CI: 0.7-1.5) of hip fracture in women with lower cheese intake (<4 portions/week) compared with women with higher cheese intake (>6 portions/week) (460). The amount of cheese per portion was not specified. Evidence from the Framingham Original Cohort showed no association between cheese intake (>1 serving/week) and hip fracture risk (RR: 0.73; 95% CI: 0.46-1.15) (453). In the Nurses' Health Study, cumulative consumption of 1 serving/day (28 g of hard cheese or cream cheese, or 120 ml of cottage or ricotta cheese) over the study period was not associated with hip fracture risk as compared to <1 serving/week of cheese (RR: 0.94; 95% CI: 0.74-1.17) (459). The meta-analysis of the combined findings from the 2 prospective studies yielded a pooled RR of incident hip fractures of 0.89 (95% CI: 0.73-1.10) for the highest cheese intake category compared with the lowest cheese intake category (**Figure 5.3**), with no evidence for heterogeneity (*P*-heterogeneity = 0.33, I^2 = 0%).

Osteoporosis as defined by BMD T-score of ≤ -2.5

Two case-control studies examined the relation between yogurt/sour cream and cheese

intake and BMD T-score ≤-2.5 in postmenopausal women (**Table 5.3**). Grgurevic et al. (461) investigated factors related to osteoporosis in postmenopausal women in Serbia (461). Cases included postmenopausal osteoporotic women with a BMD T-score ≤-2.5 at the lumbar spine and controls were age-matched (±2 years) postmenopausal women (mean age 64±9 years) with a normal BMD (lumbar spine T-score >-1.0). Yogurt and sour cream were surveyed as 1 category and there were no associations between daily consumption of yogurt and sour cream with the diagnosis of osteoporosis (461). In the same study, daily cheese consumption was associated with lower odds of osteoporosis than no daily cheese consumption (OR: 0.36; 95% CI: 0.15-0.89) (461). In the other study, Keramat et al. (456) assessed the risk factors for osteoporosis in postmenopausal women from Iran (mean age 56±6 years) and India (mean age 56±8 years). Cases, who were postmenopausal women with a BMD T-score ≤-2.5 at the lumbar spine and/or the total hip, were matched with controls from the same countries by age in 10-year age groups (456). Daily cheese consumption of ≥30 g/day was associated with lower odds of osteoporosis when compared to consumption of < 30 g/day in Iranian women (OR: 0.5; 95% CI: 0.3-0.9), but not in Indian women (no effect estimate was provided by the authors). None of the studies included in this review reported on the association of FMP consumption and change in BMD over time.

Bone turnover markers

All 3 RCTs reported the effect of either yogurt or cheese interventions on different bone turnover markers (**Table 5.4**). Heaney et al. (462) conducted a cross-over randomized trial to examine the effect of yogurt compared to a nonnutritious snack on urinary NTX. The authors reported a significant reduction in urinary NTX (-8.2 nmol bone collagen equivalents/g creatinine, P<0.03) following 7-11 days of intervention of 3 servings of yogurt daily when

compared to the consumption of a jelled fruit-flavoured snack. The amount of yogurt per serving was not specified. Bonjour et al. (463) investigated the effect of 2 servings/day of 100 g of plain cheese made from skimmed milk and fortified with vitamin D and calcium compared to no intervention over 6 weeks in 71 postmenopausal women (mean age 57±4 years). There was no significant change from baseline or difference at end of the study between the intervention and control groups in serum osteocalcin, P1NP, BSAP or CTX. However, there was a significantly greater decrease in TRACP 5b in the intervention group as compared to the control group (-0.64 U/L vs. -0.34 U/L, *P*=0.011). Johnson et al. (455) investigated the effect of 85 g of processed cheese compared with no processed cheese on osteocalcin concentrations in older women (mean age 73±7 years). Following a 2-month intervention, there were decreases in serum osteocalcin concentrations in both groups (-3.7 ng/L compared with -1.8 ng/L), but there were no differences in changes between groups (*P*=0.52).

Risk of bias and quality assessment

One of 3 trials was considered to be at low risk of bias (462). The other 2 trials had some concerns for bias arising from the lack of information on the allocation sequence and concealment of allocation (455, 463) (**Supplemental Table 5.1**). The methodological quality of the prospective cohort studies was rated as high quality (**Supplemental Table 5.2**). One case-control study was identified as of high methodological quality (461), whereas the 2 other case-control studies were identified as of moderate quality (456, 460) (**Supplemental Table 5.3**). The overall quality of evidence according to the GRADE approach for all outcomes was rated as "very low" (**Table 5.5**). The main reasons for downgrading the evidence on hip fracture risk and BMD T-score were inconsistency in FMP exposure in observational studies and high risk of bias in case-control studies related to the selection of controls not representative of the general

population of postmenopausal women. Evidence on bone turnover markers was downgraded due to high risk of bias arising from unclear allocation concealment and inconsistency in the reported markers in RCTs.

5.4 Discussion

In this systematic review and meta-analysis of prospective cohort studies, we found that a higher yogurt intake was associated with a 24% reduction in the risk of hip fracture in postmenopausal women when compared with no yogurt intake. Higher cheese consumption may be associated with reduced risk of hip fractures. However, we did not observe a significant association as our analysis was restricted to 2 studies. Similarly, limited evidence is available to confirm whether FMP intake is beneficial for other skeletal outcomes such as BMD, BMD T-score and bone turnover markers in postmenopausal women. In a recent meta-analysis of 4 cohorts of men and women, yogurt (RR 0.75, 95% CI: 0.66-0.86) and cheese consumption (RR 0.68, 95% CI: 0.61-0.77) associated with a reduced risk of hip fracture (415). In contrast to their study, we did not include the same studies because 1 study did not stratify their results by sex (464) and results from another were not stratified by age (40), and we identified an additional study that examined the relationship between FMP intake and hip fracture risk in postmenopausal women (459). Results from our analysis suggest a protective association of yogurt, but not cheese, with hip fracture risk specifically in postmenopausal women. Our study therefore adds to the literature on the potential benefits of yogurt consumption on bone health in postmenopausal women, although the low quality of evidence is low.

Findings from a case-control study that we identified during the selection process suggest that paneer, a form of cottage cheese, was associated with a reduced risk of hip fracture in Indian men and women (OR 0.152, 95% CI: 0.031-0.741) (458). The lack of association observed in our

analysis for cheese intake and hip fracture risk may possibly be explained by the relatively small number of studies (n=2) and incident fracture events to detect an association specifically in postmenopausal women. Although the heterogeneity of the studies was low, the distributions of yogurt and cheese intake levels in each cohort were dissimilar. For instance, women from the Swedish cohort (452) had higher intakes of FMP than women from the Framingham Original Cohort (453) and the Nurses' Health Study (459), as demonstrated by the reported highest intake levels (≥2 servings/day of soured milk or yogurt compared with >0 serving/week and ≥5 servings/week of yogurt, respectively). There may be a threshold effect of FMP intake on hip fracture risk, but this remains to be confirmed. Future studies must consider a standardized approach to the allocation and assessment of FMP serving sizes.

Higher yogurt consumption may also be a marker of a healthy lifestyle as it has been shown to be a reflection of long-term healthy lifestyles and dietary patterns which are positively associated with bone health (465, 466). Findings from prospective cohort studies suggest that yogurt consumers are generally more physically active, smoke less, and consume less alcohol (467, 468). Frequent yogurt consumers have also been suggested to have overall healthier eating behaviours and diet quality than infrequent consumers (465). Since such lifestyle characteristics have been shown to be protective against osteoporosis (469), it is uncertain whether the observed reduction in risk of hip fracture from the cohort studies is the result of the metabolic effects of yogurt or that of a healthier lifestyle.

The association of yogurt intakes and BMD was investigated in 4310 Irish men and women >60 years old (470). Laird et al. (470) found that BMD at the total hip and femoral neck in women were higher among those with the highest yogurt intake (>1 serving/day) compared with the lowest intake (<1 serving/week). This study was not included in our review as a result of

its cross-sectional design. We identified 1 case-control study in relation to daily yogurt consumption and osteoporosis (BMD T-score ≤-2.5) which reported no association (461). Biver et al. (471) investigated the association of FMP (included yogurts, fresh cheese, "petit-suisse" cheese, quark, and kefir) consumption on bone microstructure and BMD in 482 healthy postmenopausal women followed over 3±0.5 years. This study was not included in our analysis because the associations between FMP intake and BMD were reported as correlations and we were unable to compare extreme FMP intake levels. Nonetheless, similar to the previously mentioned study by Laird et al. (470), Biver et al. (471) found that regular (≥1 serving/day) and occasional consumers of FMPs (1-6 servings/week) had higher BMD T-scores at the lumbar spine and the total hip than non-consumers (<1 serving/week) at baseline. They observed an attenuated age-related cortical bone loss in FMP consumers, independently of total energy, calcium or protein intake, and found no association in milk or ripened cheese consumers. However, the authors found no relationship between FMP intake and the percentage annual change in BMD at the spine or total hip. Although we were unable to include studies that examined the association between kefir consumption and bone health indicators, we identified an RCT that investigated the effect of kefir-fermented milk on BMD during the full-text screening stage of the study selection process. Tu et al. (457) compared the short-term effect of kefir-fermented milk to unfermented raw milk on BMD of the spine, femoral neck, and total hip in 40 osteoporotic men and women. The average BMD increased in both groups at the end of the 6-month intervention but the changes were not significantly different between the 2 groups. Biver et al. (471) speculate that the benefits of FMP may be involved in the cortical microstructure instead of the mineralization process, but this remains to be investigated. Their study is the first to investigate the association of FMP consumption on changes of bone microstructure in

postmenopausal women and provides data to support the hypothesis that FMP may have specific metabolic effects linked to bone health compared to non-FMPs.

The association of cheese intake and BMD or BMD T-scores is less clear. In the Trinity Ulster Department of Agriculture Ageing Cohort Study, cheese intake was not associated with BMD in older women (470). Case-control studies included in the present review showed that cheese intake had either a null or protective effect against osteoporosis. The mixed findings may be explained by the difference in level of cheese intake and dietary patterns between the population groups, or the possibility of no effect to detect. The classification of all cheeses as 1 category in these studies may have also influenced the results, considering the large variety of types of cheese that differ in their fermentation process as well as their nutrient profiles.

Few RCTs were included in our systematic review and consisted of short-term interventions that examined the effect of an FMP on selected bone turnover markers. Daily yogurt intervention decreased the concentration of a bone resorption marker, urinary NTX, but the observed effect may be a result of a higher intake of calcium and protein from consuming a fruit-flavored yogurt during the intervention phase compared to the jelled fruit-flavored snack, which mainly provided carbohydrates, during the control phase of the trial (462). Nonetheless, the inverse association between FMP consumption and bone resorption markers have previously been reported in large cross-sectional studies (470, 471). In our review, cheese interventions did not have an effect bone formation marker concentrations (455, 463). One study demonstrated an effect of cheese on reducing bone resorption (463). Although TRACP 5b was significantly lower following a daily intervention of 200 g of soft cheese, it is challenging to differentiate whether the observed decrease in concentration of the bone resorption marker was the effect of cheese itself or the effect of the added calcium and vitamin D in the cheese. Moreover, whether the

observed reduction in bone resorption markers in the RCTs is clinically important or would be sustained over longer periods is unknown.

Emerging research on the cross-talk between gut microbiota and bone indicates that the gut microbiota has a major influence on bone mass and bone health. Estrogen deficiency and intestinal dysbiosis increase gut permeability, which leads to an increased production of pro-inflammatory cytokines such as TNF-α, IL-6 and, IL-1ß by immune cells in the subepithelial compartments of the intestine (472). Inflammation has well been documented to accelerate bone loss as a result of the stimulation of osteoclast formation and increased bone resorption (473). Experimental models in germ-free mice indicated that modulation of the gut microbiota with probiotics can alter intestinal permeability, influence pro-inflammatory cytokines and receptor activator of nuclear factor kappa-B ligand (RANKL) activity in the intestine and bone, leading to a decrease in osteoclast activity (471, 474). Preclinical investigations have also shown that the probiotic *Lactobacillus reuteri* ATCCPTA 6475 (*L. reuteri 6475)*, although not usually found in FMPs, prevented femur and vertebral trabecular bone volume loss and increased femoral bone density in ovariectomized mice (475, 476). Findings from a 12-month double-blind, placebo-controlled study of the probiotic *L. reuteri* 6475 in 90 women 75-80 y with low BMD demonstrated reduced loss of tibia total volumetric BMD in the intervention group (477), whereas there was no difference in the markers of inflammation (C-reactive protein and TNF-α). Given that FMPs are predominant sources of different strains of probiotics in the diet, investigation into the effect FMP on a wider panel of pro-inflammatory cytokines including TNF-α, IL-6, IL-1ß, and RANKL may provide a better understanding of the mechanism of action of FMP on skeletal health in postmenopausal women.

The present study has some limitations. For example, we were unable to compare the

effect of FMP and non-FMP on bone health indicators in postmenopausal women. The question regarding whether FMPs exert more beneficial effects than non-FMPs on bone health remains unclear. Moreover, cheeses and yogurts were surveyed as 2 generalized groups in the included observational studies. Given that cheeses are produced from a variety of fermentation processes and that live cultures are not found in all cheeses, there is a possibility that each variety may contribute to bone health differently. Different types of cheese were used in the included RCTs, such as soft plain cheese (463) and processed cheese (455), resulting in a challenge to compare outcomes across studies considering the dissimilarities in the preparation and processing of the cheeses as well as their different nutrient profiles. Similarly, none of the identified studies in our review specifically examined the strains of probiotics found in yogurts, or other types of FMP such as Greek-style yogurts or kefir, which have higher protein content than yogurt. Hence, comprehension of the beneficial contribution of live bacteria and that of the food matrix in FMPs on bone health, in combination or separately, requires further exploration. Our study was also limited by the difference in categorization of intakes and lack of detail on serving sizes from some studies, which made it difficult to compare the results across studies. Hence, our analysis primarily considered the highest compared with the lowest exposure category of FMP. Another limitation would be recall bias related to the differential reporting of FMP intake between cases and controls in case-control studies. For example, cases may recall lower intake of FMP than controls when reporting their past food intake and hence introducing bias to the effect estimate. Finally, we did not include studies that reported data on volumetric BMD by peripheral quantitative computed tomography because of the different parameters of bone and use of appendicular sites and we wished to consider measurements used in clinical practice. In addition, most of the studies included in this systematic review are observational studies and causal

relations cannot be inferred. Further research is required to confirm our findings and to provide more robust evidence on the potential role of each type of FMP on bone health in postmenopausal women.

Conclusion

Evidence from prospective cohort studies suggest that greater consumption of FMP in the form of yogurt is associated with a reduced risk of hip fracture in postmenopausal women compared with low or no intake, albeit the quality of evidence is very low. Daily cheese consumption may be protective against osteoporosis, but more studies are required to confirm this association. From a public health perspective, more rigorously designed RCTs are required to guide dietary guidelines regarding whether to promote FMPs over milk products overall for bone health in postmenopausal women.

Table 5.1 Characteristics of studies that examined the association between yogurt consumption and hip fractures in postmenopausal women[1]

Author (reference)	Design (Cohort name)	*N*	*n* cases			Age, y	Duration of follow-up, y	Intake categories[2]	RR (95% CI)	Adjustments
			Total	Highest intake group	Lowest intake group					
Sahni et al. (453)[3]	Prospective cohort (Framingham Original Cohort)	469	76	19/113	57/356	77 ± 4.8	11.6	None vs. >0 serving/wk	1.12 (0.66-1.91)	Age, BMI, height, total energy intake, current smoking, calcium supplements, vitamin D supplements
Feskanich et al. (459)	Prospective cohort (Nurses' Health Study)	80,600	2138	32/49 p-y	668/560 p-y	54 (range: 34-60)	20.8	None vs. ≥5 servings/wk	0.77 (0.53-1.12)	Age, follow-up cycle, total energy intake, calcium and vitamin D from non-dairy foods plus supplements, protein from non-dairy foods, retinol from supplements, vitamin D, caffeine, alcohol, milk during teenage years, BMI, height, physical activity, smoking, use of postmenopausal hormones, use of thiazide diuretics, furosemide-type diuretics and oral steroids, and diagnoses of cancer, diabetes and cardiovascular disease, milk and cheese intakes.
Michaelsson et al. (452)[3]	Prospective cohort (Swedish Mammography Cohort)	27150	4,777	451/ 41 p-y	1446/ 136 p-y	63 ± 5.2	22	None vs. ≥2 servings/d	0.71 (0.63-0.79)	Age, BMI, height, energy intake, alcohol intake, milk and cheese intake, fruit and vegetable intake, red and processed meat intake, education, cohabitating status, physical activity, smoking habits, ever use of antioxidant-containing supplements, Charlson's weighted comorbidity index.

[1] p-y, person-years (in thousands).
[2] Intake categories: Sahni et al. (453), 1 serving = 1 cup or 240 mL. Feskanich et al. (459), 1 serving = 1 cup or 240 mL. Michaelsson et al. (452), yogurt and sour milk were assessed together, 1 serving = 200 mL.
[3] Data for postmenopausal or women ≥55 years only were obtained from authors.

Table 5.2 Characteristics of studies that examined the association between cheese consumption and hip fractures in postmenopausal women[1]

Author (reference)	Design (Participant characteristics)	*n*	*n* cases			Age, y	Duration of follow-up, y	Intake categories[2]	OR/RR (95% CI)	Adjustments
			Total	Highest intake group	Lowest intake group					
Tavani et al. (460)	Case-control (hospitalized patients)	960	241	---	---	64 (range: 45-74)	---	<4 portions/wk vs. >6 portions/wk	OR: 1.0 (0.7-1.5)	Age, education, BMI, smoking status, total alcohol consumption, and estrogen therapy
Sahni et al. (453)[3]	Prospective cohort (Framingham Original Cohort)	469	76	36/259	40/210	77±4.8	11.6	≤1 serving/wk vs. >1 serving/wk	RR: 0.73 (0.46-1.15)	Age, BMI, height, total energy intake, current smoking, calcium supplements, vitamin D supplements
Feskanich et al. (459)	Prospective cohort (Nurses' Health Study)	80,600	2138	279/261 p-y	126/112 p-y	54 (range: 34-60)	20.8	<1 serving/wk vs. ≥1 serving/d	RR: 0.94 (0.74-1.17)	Age, follow-up cycle, total energy intake, calcium and vitamin D from non-dairy foods plus supplements, protein from non-dairy foods, retinol from supplements, vitamin D, caffeine, alcohol, milk during teenage years, BMI, height, physical activity, smoking, use of postmenopausal hormones, use of thiazide diuretics, furosemide-type diuretics and oral steroids, and diagnoses of cancer, diabetes and cardiovascular disease, milk and cheese intakes.

[1] p-y, person-years (in thousands).
[2] Intake categories: Tavani et al. (460), portion size was not described by the authors. Sahni et al .(453), cheese intake was calculated as the combined intake of cottage/ricotta cheese (1 serving = 0.5 cup or 120 mL) and American cheese (1 slice or 1 oz or 28 g) and other cheeses. Feskanich et al. (459), cheese intake was calculated as the combined intake of hard and soft cheeses (1 serving = 1 oz or 28 g), and cottage/ricotta cheese (1 serving = 0.5 cup or 120 mL).
[3] Data for postmenopausal or women ≥55 years only were obtained from authors.

Table 5.3 Characteristics of studies that examined the association of fermented milk product consumption (cheese or yogurt) with bone mineral density T-score[1]

Author (reference)	Study population	Age, y	Study period	Bone health outcome	Cases, *n*	Controls, *n*	FMP	Intake categories	OR (95% CI)	Matched/Adjusted variables
Grgurevic et al. (461)	Outpatients	64±9.0	2006-2007	BMD T-score ≤-2.5 (lumbar spine)	100	100	Yogurt, sour cream	No daily consumption vs. Daily consumption	NR[2]	NR
Grgurevic et al. (461)	Outpatients	64±9.0	2006-2007	BMD T-score ≤-2.5 (lumbar spine)	100	100	Cheese	No daily consumption vs. Daily consumption	0.36 (0.15-0.89)	Body weight < 65 kg, thin constitution in childhood, history of previous fracture, family history of fracture, age at menopause < 47 y, fish consumption
Keramat et al. (456)	Outpatients (Iran)	57±6.6	2002-2005	BMD T-score ≤-2.5 (lumbar spine and/or total hip)	178	185	Cheese	<30 g/d vs. ≥30 g/day	0.5 (0.3-0.9)	Age, height, and weight
Keramat et al. (456)	Outpatients (India)	58±7.8	2002-2005	BMD T-score ≤-2.5 (lumbar spine and/or total hip)	203	151	Cheese	<30 g/d vs. ≥30 g/day	NR[3]	Age, height, and weight

[1] BMD, bone mineral density; FMP, fermented milk product; NR, not reported.
[2] No data was available for the effect estimate. Grgurevic et al. (461) reported that daily consumption of yogurt/sour cream was no long significantly (P>0.05) associated with osteoporosis in multiple forward conditional logistic regression. The unadjusted OR for daily yogurt/sour cream consumption was 0.45 (0.25-0.82).
[3] No data was available for the effect estimate. Keramat et al. (456) reported the OR as 'non-significant' (P>0.05).

Table 5.4 Characteristics of randomized controlled trials that assessed the impact of fermented milk product (cheese or yogurt) consumption on bone turnover markers[1]

Author, (reference)	*n*	Participant characteristics	Age, y	Design	Intervention[2]	Control	Duration	Bone formation markers	Bone resorption marker	Reported results
Heaney et al. (462)	29	All white; usual calcium intake <600 mg; BMI: 27.3±3.9 kg/m^2	61±4.3	Cross-over	3 servings/d of fruit-flavored yogurt (*n*=29)	3 servings/d of jelled fruit-flavored snack (*n*=29)	7-11 days	N/A	NTX	-8.2 nmol BCE/g creatinine (22% lower than control)
Bonjour et al. (463)	71	Postmenopausal ≥3 y; usual calcium intake <600 mg; BMI : 22.9±2.5 and 23.1±2.2	57±3.9	Parallel	200 g of skimmed-milk, soft, plain cheese (*n*=36)	Usual diet (*n*=35)	6 weeks	BAP, OC, PINP	CTX, TRACP 5b	Significant decrease in TRACP 5b in both groups and the decline was greater in the treated group vs the control group (-0.64 vs. -0.34; *P* = 0.011)
Johnson et al. (455)[3]	46	Usual calcium intake >1000 mg/d	73±7.0	Parallel	85 g of processed cheese (*n*=23)	No processed cheese (*n*=23)	2 months	OC	N/A	No difference in change of OC concentrations between groups

[1] BAP, bone alkaline phosphatase; BCE, bone collagen equivalents; CTX, C-terminal telopeptide of type I collagen; FMP, fermented milk product; N/A, not available; NTX, N-telopeptide of type I collagen; OC, osteocalcin; PINP, procollagen type I propeptides; TRACP 5b, tartrate-resistant acid phosphatase 5b.
[2] Intervention: Heaney et al. (462), serving size of yogurt was not specified by the authors. Bonjour et al. (463), 2 x 100 g of skimmed milk, soft, plain cheese fortified with 1.25 µg vitamin D and total calcium content of 200 mg per 100 g. Johnson et al. (455), as shown in the table.
[3] Data for female participants were obtained from authors.

Table 5.5 GRADE assessment of the quality of evidence for fermented milk product consumption on bone health outcomes in postmenopausal women[1]

FMP	Outcome	Study design, *n*	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Quality
Yogurt	Hip fracture	3 prospective cohort studies	Not serious	Serious inconsistency[3]	No serious indirectness	No serious imprecision	Undetected[2]	Very low
	BMD T-score ≤ -2.5	1 case-control study	Serious[4]	Not relevant[5]	No serious indirectness	No serious imprecision	Undetected[2]	Very low
	Bone turnover markers	1 RCT	Not serious	Not relevant[5]	Serious indirectness[6]	Serious imprecision[7]	Undetected[2]	Very low
Cheese	Hip fracture	2 prospective cohort studies	Not serious	Serious inconsistency[8]	No serious indirectness	Serious imprecision[9]	Undetected[2]	Very low
	BMD T-score ≤ -2.5	2 case-control studies	Serious[10]	Serious inconsistency[11]	No serious indirectness	Serious imprecision[12]	Undetected[2]	Very low
	Bone turnover markers	2 RCTs	Serious[13]	Serious inconsistency[14]	Serious indirectness[15]	Serious imprecision[16]	Undetected[2]	Very low

[1] BMD, bone mineral density ; FMP, fermented milk product, RCT, randomized controlled trial.
[2] Publication bias: There were less than 10 studies for each outcome.
[3] Serious inconsistency: Although statistical tests suggested no heterogeneity across studies, there was inconsistency in exposure comparisons in the highest intake categories across all 3 studies (i.e., > 0 serving/week vs. ≥ 5 servings/week vs. ≥ 2 servings/day).
[4] Serious risk of bias: The controls were hospital patients which are not considered representative of the general population of postmenopausal women.
[5] Inconsistency category not relevant: Only one study reported on this outcome.
[6] Serious indirectness: The comparison used in this study was not a milk product and had a very different nutrient profile than the intervention, which would have influenced the outcome.
[7] Serious imprecision: Small sample size.
[8] Serious inconsistency: Although statistical tests suggest no heterogeneity across studies, there was inconsistency in exposure comparison in the highest intake categories (i.e., > 1 serving/week vs. ≥ 1 serving/day).
[9] Serious imprecision: The optimal information size is met, but confidence interval overlaps no effect and the confidence interval did not exclude important benefit (i.e., lower bound of confidence interval = 0.73, so > 25% for largest plausible effect).
[10] Serious risk of bias: Missing data and potential confounders not adjusted for in the study by Keramat et al. (456), and the controls were hospital patients in the study by Grgurevic et al. (461) which are not considered representative of the general population of postmenopausal women.
[11] Serious inconsistency: The effect size is missing for one of the sub-populations by Keramat et al. (456) and we deduced that the confidence intervals would have less overlapping.
[12] Serious imprecision: Large confidence interval.
[13] Serious risk of bias: Unclear allocation concealment in the two studies and imbalance in vitamin D status at baseline between study groups in one study.
[14] Serious inconsistency: The two studies reported on different bone turnover markers and the interventions were different across studies.
[15] Serious indirectness: The comparison used in the two studies was a usual diet, which did not allow a direct comparison to the intervention and would have influenced the outcome.
[16] Serious imprecision: Small sample size

Figure 5.1 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram of studies through the review process for to the selection of studies for the systematic review and meta-analysis of studies of fermented milk product consumption and bone health in postmenopausal women.

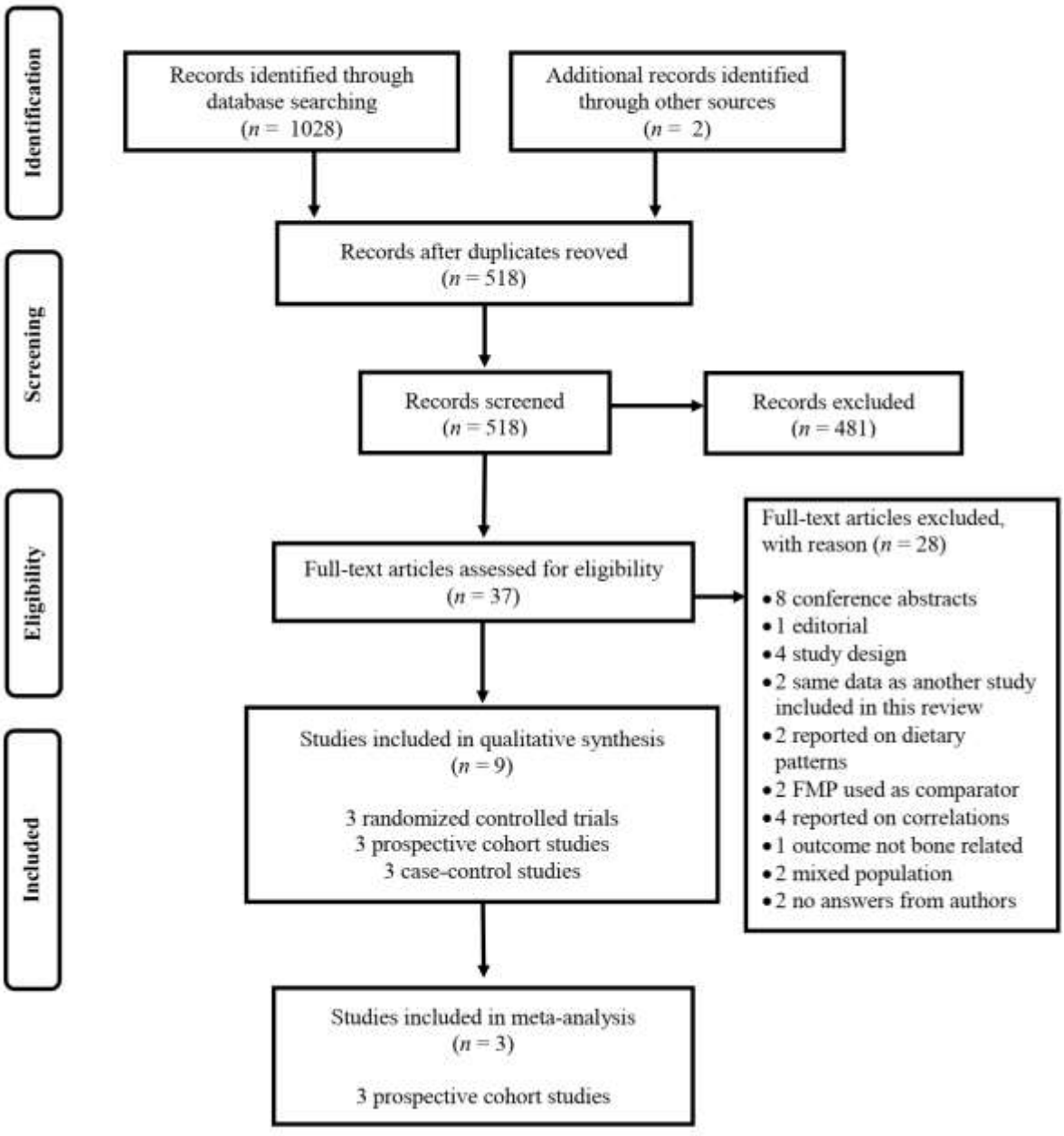


Figure 5.2 Random-effects (RE) model meta-analysis of prospective studies on yogurt consumption (highest vs. lowest intake levels) and risk of hip fractures in postmenopausal women.

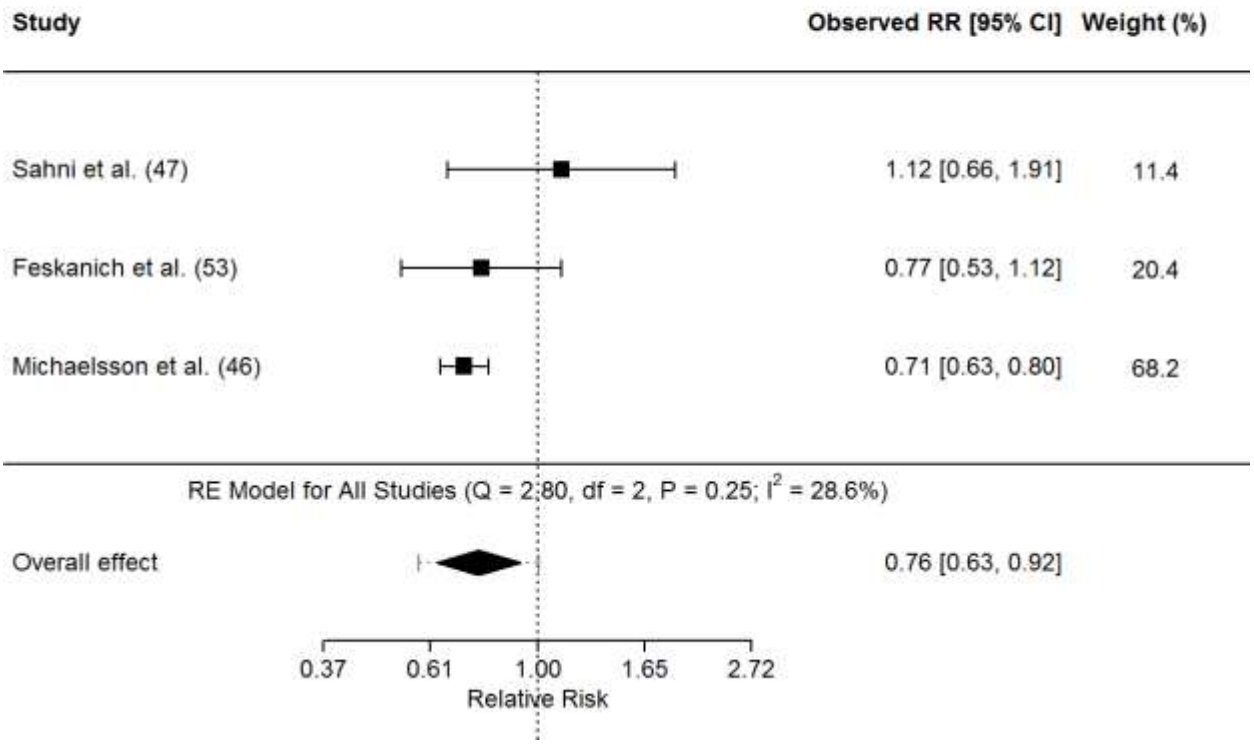
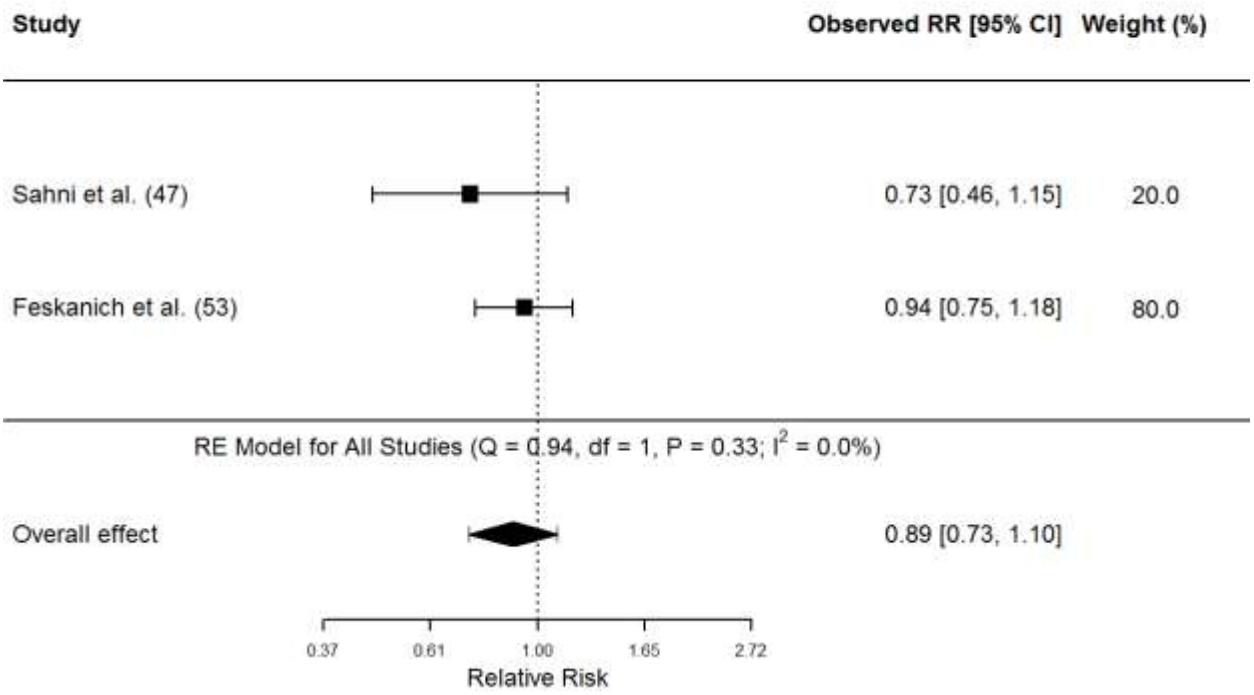


Figure 5.3 Random-effects (RE) model meta-analysis of prospective studies on cheese consumption (highest vs. lowest intake levels) and risk of hip fractures in postmenopausal women



Online Supporting Material

Fermented milk products and bone health in postmenopausal women: a systematic review of randomized controlled trials, prospective cohorts and case-control studies[1-3]

Authors

Angel M. Ong[1,2], Kai Kang[1, 3], Hope A. Weiler[1], Suzanne N. Morin[2,4]

Affiliations

[1] School of Human Nutrition, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

[2] Research Institute of the McGill University Health Centre, 5252 de Maisonneuve Ouest, Montreal, Quebec, Canada, H4A 3S5

[3] Department of Research and Surveillance Evaluation, Shanghai Center for Health Promotion, 358 Jiaozhou Road, Jingan Area, Shanghai, China, 200040

[4] Department of Medicine, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada, H3G 1Y6

Corresponding author:

Dr. Suzanne N. Morin

Montreal General Hospital

1650 Cedar Avenue, room B2-118

Montreal (QC) Canada H3G 1A4

Telephone number: (514)937-7298

Email address: suzanne.morin@mcgill.ca

Supplemental Methods

Search strategies by electronic database to retrieve literature

Database: MEDLINE

1. exp Cultured Milk Products/

2. Milk/ and (ferment* or culture*).tw,kf.

3. Dairy Products/ and (ferment* or culture*).tw,kf.

4. (ferment* adj3 (milk or dair*)).ti,ab,kf.

5. (cultured adj3 (milk or dair*)).ti,ab,kf.

6. (yoghurt or yogourt or yoghourt or yogurt or zabadi).ti,ab,kf.

7. cheese*.ti,ab,kf.

8. (buttermilk or butter-milk).ti,ab,kf.

9. (koumis* or kumis* or kumys).ti,ab,kf.

10. kefir.ti,ab,kf.

11. acidophilus milk.ti,ab,kf.

12. viili.ti,ab,kf.

13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12

14. Bone Density/

15. ((bone adj3 density) or osseous density or (bone adj3 content)).ti,ab,kf.

16. exp Osteoporosis/

17. osteoporo*.ti,ab,kf.

18. exp Fractures, Bone/

19. fracture*.ti,ab,kf.

20. exp Bone Remodeling/

21. (bone and (remodeling or remodelling or re-modeling or re-modelling)).ti,ab,kf.

22. (bone adj2 (loss or lost or resorpt*)).ti,ab,kf.

23. (bone turnover or bone turn-over).ti,ab,kf.

24. Osteocalcin/

25. (osteocalcin or (bone adj3 carboxyglutamic) or bone ajd3 carboxy-glutamic or (bone adj3 GLA)).ti,ab,kf.

26. Alkaline Phosphatase/

27. ((bone adj3 alkaline phosphatase) or BAP or BALP or BSAP).ti,ab,kf.

28. (((N-terminal propeptide or N-terminal pro-peptide or amino-terminal propeptide or amino-terminal pro-peptide) adj3 (type 1 collagen or type I collagen)) or PINP or P1NP).ti,ab,kf.

29. (((C-terminal propeptide or C-terminal pro-peptide or carboxy*-terminal propeptide or carboxy*-terminal pro-peptide) adj3 (type 1 collagen or type I collagen)) or PICP or P1CP).ti,ab,kf.

30. (hydroxyproline or hydroxy-proline or PYD).ti,ab,kf.

31. (deoxypyridonoline or DPD).ti,ab,kf.

32. (((C-terminal or carboxy*-terminal) adj5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or CTX).ti,ab,kf.

33. (((N-terminal or amino-terminal) adj5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or NTX).ti,ab,kf.

34. ICTP.ti,ab,kf.

35. Tartrate-Resistant Acid Phosphatase/

36. ((tartrate-resistant acid phosphatase or TRACP) adj2 (5b or type-5)).ti,ab,kf.

37. ((bone or skelet*) adj3 health).ti,ab,kf.

38. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37

39. 13 and 38

40. Animals/ not (Animals/ and Humans/)

41. ((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,jw.

42. 40 or 41

43. 39 not 42

Database: Embase

1. exp fermented milk product/

2. Milk/ and (ferment* or culture*).tw,kw.

3. Dairy Product/ and (ferment* or culture*).tw,kw.

4. cheese/

5. (ferment* adj3 (milk or dair*)).ti,ab,kw.

6. (cultured adj3 (milk or dair*)).ti,ab,kw.

7. (yoghurt or yogourt or yoghourt or yogurt or zabadi).ti,ab,kw.

8. cheese*.ti,ab,kw.

9. (buttermilk or butter-milk).ti,ab,kw.

10. (koumis* or kumis* or kumys).ti,ab,kw.

11. kefir.ti,ab,kw.

12. acidophilus milk.ti,ab,kw.

13. viili.ti,ab,kw.

14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13

15. exp bone density/

16. ((bone adj3 density) or osseous density or (bone adj3 content)).ti,ab,kw.

17. exp osteoporosis/

18. osteoporo*.ti,ab,kw.

19. exp fracture/

20. fracture*.ti,ab,kw.

21. bone remodeling/

22. (bone and (remodeling or remodelling or re-modeling or re-modelling)).ti,ab,kw.

23. osteolysis/

24. (bone adj2 (loss or lost or resorpt*)).ti,ab,kw.

25. bone turnover/

26. (bone turnover or bone turn-over).ti,ab,kw.

27. osteocalcin/

28. (osteocalcin or (bone adj3 carboxyglutamic) or (bone adj3 carboxy-glutamic acid) or (bone adj3 GLA)).ti,ab,kw.

29. alkaline phosphatase bone isoenzyme/

30. ((bone adj3 alkaline phosphatase) or BAP or BALP or BSAP).ti,ab,kw.

31. (((N-terminal propeptide or N-terminal pro-peptide or amino-terminal propeptide or aminoterminal propeptide) adj3 (type 1 collagen or type I collagen)) or PINP or P1NP).ti,ab,kw.

32. (((C-terminal propeptide or C-terminal pro-peptide or carboxy*-terminal propeptide or carboxy*-terminal pro-peptide) adj3 (type 1 collagen or type I collagen)) or PICP or P1CP).ti,ab,kw.

33. (hydroxyproline or hydroxy-proline or PYD).ti,ab,kw.

34. (deoxypyridinoline or DPD).ti,ab,kw.

35. (((C-terminal or carboxy*-terminal) adj5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or CTX).ti,ab,kw.

36. (((N-terminal or amino-terminal) adj5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or NTX).ti,ab,kw.

37. ICTP.ti,ab,kw.

38. acid phosphatase tartrate resistant isoenzyme/

39. ((tartrate-resistant acid phosphatase or TRACP) adj2 (5b or type-5)).ti,ab,kw.

40. ((bone or skelet*) adj3 health).ti,ab,kw.

41. 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40

42. 14 and 41

43. (Animal experiment/ or experimental animal/) not Human/

44. ((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,jx.

45. 43 or 44

46. 42 not 45

Database: PubMed

(("fermented milk"[Text Word] OR "fermented milks"[Text Word] OR "fermented dairy"[Text Word] OR "cultured milk"[Text Word] OR "cultured milks"[Text Word] OR "cultured dairy"[Text Word]) OR (yoghurt[Text Word] OR yogourt[Text Word] OR yoghourt[Text Word]

OR yogurt[Text Word] OR zabadi[Text Word]) OR (cheese*[Text Word]) OR (buttermilk[Text Word] OR "butter-milk"[Text Word]) OR (koumis*[Text Word] OR kumis*[Text Word] OR kumys[Text Word]) OR (kefir[Text Word]) OR ("acidophilus-milk"[Text Word]) OR (viili[Text Word])) AND (("bone density"[Text Word] OR "bone mineral density"[Text Word] OR "bone mineral content"[Text Word] OR "osseous density"[Text Word]) OR (osteoporo*[Text Word]) OR (fracture*[Text Word]) OR ("bone remodeling"[Text Word] OR "bone remodelling"[Text Word] OR "bone re-modeling"[Text Word] OR "bone re-remodelling"[Text Word]) OR ("bone resorption"[Text Word] OR "resorption of bone"[Text Word] OR "bone loss"[Text Word] OR "bone lost"[Text Word]) OR ("bone turnover"[Text Word] OR "bone turn-over"[Text Word]) OR (osteocalcin[Text Word] OR "bone gamma-carboxyglutamic acid-containing protein"[Text Word] OR "bone gamma-carboxyglutamic acid protein"[Text Word] OR "bone GLA"[Text Word]) OR ("bone alkaline phosphatase"[Text Word] OR "bone specific alkaline phosphatase"[Text Word] OR BAP[Text Word] OR BALP[Text Word] OR BSAP[Text Word]) OR ("N-terminal propeptide of type 1 collagen"[Text Word] OR "N-terminal pro-peptide of type 1 collagen"[Text Word] OR "N-terminal propeptide of type I collagen"[Text Word] OR "N-terminal pro-peptide of type I collagen"[Text Word] OR "amino-terminal propeptide of type 1 collagen"[Text Word] OR "amino-terminal pro-peptide of type 1 collagen"[Text Word] OR "amino-terminal propeptide of type I collagen"[Text Word] OR "amino-terminal pro-peptide of type I collagen"[Text Word] OR PINP[Text Word] OR P1NP[Text Word]) OR ("C-terminal propeptide of type 1 collagen"[Text Word] OR "C-terminal pro-peptide of type 1 collagen"[Text Word] OR "C-terminal propeptide of type I collagen"[Text Word] OR "C-terminal pro-peptide of type I collagen"[Text Word] OR "carboxy-terminal propeptide of type 1 collagen"[Text Word] OR "carboxy-terminal pro-peptide of type 1 collagen"[Text Word] OR "carboxy-terminal

propeptide of type I collagen"[Text Word] OR "carboxy-terminal pro-peptide of type I collagen"[Text Word] OR "carboxyl-terminal propeptide of type 1 collagen"[Text Word] OR "carboxyl-terminal pro-peptide of type 1 collagen"[Text Word] OR "carboxyl-terminal propeptide of type I collagen"[Text Word] OR "carboxyl-terminal pro-peptide of type I collagen"[Text Word] OR PICP[Text Word] OR P1CP[Text Word]) OR (hydroxyproline[Text Word] OR hydroxy-proline[Text Word] OR PYD[Text Word]) OR (deoxypyridinoline[Text Word] OR DPD[Text Word]) OR ("C-terminal of type 1 collagen"[Text Word] OR "C-terminal of type I collagen"[Text Word] OR "C-terminal of collagen type 1"[Text Word] OR "C-terminal of collagen type I"[Text Word] OR "carboxy-terminal of type 1 collagen"[Text Word] OR "carboxy-terminal of type I collagen"[Text Word] OR "carboxy-terminal of collagen type 1"[Text Word] OR "carboxy-terminal of collagen type I"[Text Word]
OR "carboxyl-terminal of type 1 collagen"[Text Word] OR "carboxyl-terminal of type I collagen"[Text Word] OR "carboxyl-terminal of collagen type 1"[Text Word] OR "carboxyl-terminal of collagen type I"[Text Word] OR CTX[Text Word]) OR ("N-terminal of type 1 collagen"[Text Word] OR "N-terminal of type I collagen"[Text Word] OR "N-terminal of collagen type 1"[Text Word] OR "N-terminal of collagen type I"[Text Word] OR "amino-terminal of type 1 collagen"[Text Word] OR "amino-terminal of type I collagen"[Text Word] OR "amino-terminal of collagen type 1"[Text Word] OR "amino-terminal of collagen type I"[Text Word] OR NTX[Text Word]) OR (ICTP[Text Word]) OR ("tartrate-resistant acid phosphatase"[Text Word] OR "tartrate resistant acid phosphatase"[Text Word] OR "TRACP-5b"[Text Word] OR TRACP5b[Text Word] OR "TRACP type 5"[Text Word] OR "TRACP type-5"[Text Word]) OR ("bone health"[Text Word] OR "skeletal health"[Text Word]))

Database: Cumulative Index to Nursing and Allied Health Literature

S43 S39 NOT S42

S42 S40 OR S41

S41 TI ((animal OR animals OR canine* OR cat OR cats OR dog OR dogs OR feline OR hamster* OR mice OR monkey OR monkeys OR mouse OR murine OR pig OR pigs OR piglet* OR porcine OR primate* OR rabbit* OR rats OR rat OR rodent* OR sheep* OR veterinar*) NOT (human* or patient*))

S40 (MH "Animals") NOT ((MH "Animals") AND (MH "Humans))

S39 S13 AND S38

S38 S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37

S37 (AB ((bone OR skelet*) N3 health) OR (TI ((bone OR skelet*) N3 health))

S36 (AB ((tartrate-resistant acid phosphatase OR TRACP) N2 (5b OR type-5)) OR (TI ((tartrate-resistant acid phosphatase OR TRACP) N2 (5b OR type-5)))

S35 (MH "Tartrate-Resistant Acid Phosphatase")

S34 (AB (ICTP) OR (TI ICTP))

S33 (AB (((N-terminal OR amino-terminal) N5 (type 1 collagen OR type I collagen OR collagen type 1 OR collagen type I)) OR NTX) OR (TI (((N-terminal OR amino-terminal) N5 (type 1 collagen OR type I collagen OR collagen type 1 OR collagen type I)) OR NTX))

S32 (AB (((C-terminal OR carboxy*-terminal) N5 (type 1 collagen OR type I collagen OR collagen type 1 OR collagen type I)) OR CTX) OR (TI (((C-terminal OR carboxy*-terminal) N5 (type 1 collagen OR type I collagen OR collagen type 1 OR collagen type I)) OR CTX))

S31 (AB (deoxypyridonoline OR DPD) OR (TI (deoxypyridonoline OR DPD))

S30 (AB (hydroxyproline OR hydroxy-proline OR PYD) OR (TI (hydroxyproline OR hydroxy-proline OR PYD))

S29 (AB (((C-terminal propeptide OR C-terminal pro-peptide OR carboxy*-terminal propeptide OR carboxy*-terminal pro-peptide) N3 (type 1 collagen OR type I collagen)) OR PICP OR P1CP) OR (TI (((C-terminal propeptide OR C-terminal pro-peptide OR carboxy*-terminal propeptide OR carboxy*-terminal pro-peptide) N3 (type 1 collagen OR type I collagen)) OR PICP OR P1CP))

S28 (AB (((N-terminal propeptide OR N-terminal pro-peptide OR amino-terminal propeptide OR amino-terminal pro-peptide) N3 (type 1 collagen OR type I collagen)) OR PINP OR P1NP) OR (TI (((N-terminal propeptide OR N-terminal pro-peptide OR amino-terminal propeptide OR amino-terminal pro-peptide) N3 (type 1 collagen OR type I collagen)) OR PINP OR P1NP))

S27 (AB ((bone N3 alkaline phosphatase) OR BAP OR BALP OR BSAP) OR (TI ((bone N3 alkaline phosphatase) OR BAP OR BALP OR BSAP))

S26 (MH "Alkaline Phosphatase")

S25 (AB (osteocalcin OR (bone N3 carboxyglutamic) OR bone N3 carboxy-glutamic OR (bone N3 GLA)) OR (TI(osteocalcin OR (bone N3 carboxyglutamic) OR bone N3 carboxy-glutamic OR (bone N3 GLA)))

S24 (MH "Osteocalcin")

S23 (AB (bone turnover OR bone turn-over) OR (TI (bone turnover OR bone turn-over))

S22 (AB (bone N2 (loss OR lost OR resorpt*)) OR (TI (bone N2 (loss OR lost OR resorpt*)))

S21 (AB (bone AND (remodeling OR remodelling OR re-modeling OR re-modelling)) OR (TI (bone AND (remodeling OR remodelling OR re-modeling OR re-modelling)))

S20 (MH "Bone Remodeling+")

S19 (AB (fracture*) OR (TI (fracture*))

S18 (MH "Fractures+")

S17 (AB (osteoporo*) OR (TI (osteoporo*))

S16 (MH "Osteoporosis+")

S15 (AB ((bone N3 density) OR "osseous density" OR (bone N3 content)) OR (TI ((bone N3 density) OR "osseous density" OR (bone N3 content)))

S14 (MH "Bone Density")

S13 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12

S12 (AB (viili) OR (TI (viili))

S11 (AB ("acidophilus milk") OR (TI ("acidophilus milk"))

S10 (AB (kefir) OR (TI (kefir))

S9 (AB (koumis* OR kumis* OR kumys) OR (TI(koumis* OR kumis* OR kumys))

S8 (AB (buttermilk OR butter-milk) OR (TI (buttermilk OR butter-milk))

S7 (AB (cheese*) OR (TI (cheese*))

S6 (AB (yoghurt OR yogourt OR yoghourt OR yogurt OR zabadi) OR (TI (yoghurt OR yogourt OR yoghourt OR yogurt OR zabadi)))

S5 (AB (cultured N3 (milk OR dair*)) OR (TI (cultured N3 (milk OR dair*)))

S4 (AB (ferment* N3 (milk OR dair*)) OR (TI (ferment* N3 (milk OR dair*)))

S3 (MH "Dairy Products+") AND (TX (ferment* OR culture*))

S2 MH "milk" AND TX (ferment* OR culture*)

S1 (MH "Cultured Milk Products+")

Database: Cochrane Central Register of Controlled Trials

#1 (ferment* near/3 (milk or dair*)):ti,ab,kw

#2 (cultured near/3 (milk or dair*)):ti,ab,kw

#3 (yoghurt or yogourt or yoghourt or yogurt or zabadi):ti,ab,kw

#4 cheese*:ti,ab,kw

#5 (buttermilk or butter-milk):ti,ab,kw

#6 koumis* or kumis* or kumys:ti,ab,kw

#7 kefir:ti,ab,kw

#8 acidophilus-milk:ti,ab,kw

#9 viili:ti,ab,kw

#10 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9

#11 ((bone near/3 density) or osseous density or (bone near/3 content)):ti,ab,kw

#12 osteoporo*:ti,ab,kw

#13 fracture*:ti,ab,kw

#14 (bone and (remodeling or remodelling or re-modeling or re-modelling)):ti,ab,kw

#15 (bone near/2 (loss or lost or resorpt*)):ti,ab,kw

#16 (bone turnover or bone turn-over):ti,ab,kw

#17 (osteocalcin or (bone near/3 carboxyglutamic) or (bone near/3 carboxy-glutamic acid) or (bone near/3 GLA)):ti,ab,kw

#18 ((bone near/3 alkaline phosphatase) or BAP or BALP or BSAP):ti,ab,kw

#19 (((N-terminal propeptide or N-terminal pro-peptide or amino-terminal propeptide or amino-terminal propeptide) near/3 (type 1 collagen or type I collagen)) or PINP or P1NP):ti,ab,kw

#20 (((C-terminal propeptide or C-terminal pro-peptide or carboxy*-terminal propeptide or carboxy*-terminal pro-peptide) near/3 (type 1 collagen or type I collagen)) or PICP or P1CP):ti,ab,kw

#21 (hydroxyproline or hydroxy-proline or PYD):ti,ab,kw

#22 (deoxypyridinoline or DPD):ti,ab,kw

#23 (((C-terminal or carboxy*-terminal) near/5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or CTX):ti,ab,kw

#24 (((N-terminal or amino-terminal) near/5 (type 1 collagen or type I collagen or collagen type 1 or collagen type I)) or NTX):ti,ab,kw

#25 ICTP:ti,ab,kw

#26 ((tartrate-resistant acid phosphatase or TRACP) near/2 (5b or type-5)):ti,ab,kw

#27 ((bone or skelet*) near/3 health):ti,ab,kw

#28 #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27

#29 #10 and #28

Supplemental Tables 5.1-5.3

Supplemental Table 5.1 Risk of bias assessment of included randomized controlled trials[1]

References	Domain A	Domain B	Domain C	Domain D	Domain E	Overall risk of bias
Heaney, 2002 (462)	Low	Low	Low	Low	Low	Low
Bonjour, 2012 (463)	Some concerns	Low	Low	Low	Low	Some concerns
Johnson, 2005 (455)	Some concerns	Low	Low	Low	Low	Some concerns

[1] Domains: (A) bias arising from the randomisation process, (B) bias owing to deviations from intended interventions, (C) bias owing to missing outcome data, (D) bias in measurement of the outcome, (E) bias in selection of the reported result. The overall risk of bias grade was calculated by assessing the five domains [A–E].

Supplemental Table 5.2 Methodological quality of prospective cohort studies included in the systematic review using the Newcastle-Ottawa Scale[1]

First author, publication year (reference)	Selection				Comparability	Outcome			Total quality scores
	Representativeness of the exposed cohort	Selection of the unexposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study	Control for important factor or additional factor[2]	Assessment of outcome	Follow-up long enough for outcomes to occur [3]	Adequacy of follow-up of cohorts[4]	
Sahni, 2014 (453)	✸	✸	✸	✸	✸✸	✸	✸	✸	9
Feskanich, 2018 (459)	✸	✸	✸	✸	✸✸	-	✸	✸	8
Michaelsson, 2017 (452)	✸	✸	✸	-	✸✸	✸	✸	✸	8

[1] A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor.
[2] A maximum of 2 stars could be awarded for this item. Studies that controlled for calcium and vitamin D supplement use received one star, whereas studies that controlled for other important confounders (smoking, hormone replacement therapy or energy intake) received an additional star.
[3] A cohort study with a follow-up time ≥5 years for incident hip fractures or ≥2 years for change in BMD was assigned one star.
[4] A cohort study with a follow-up rate >90% was assigned one star.

Supplemental Table 5.3 Methodological quality of case-control studies included in the systematic review using the Newcastle-Ottawa Scale[1]

First author, publication year (reference)	Selection				Comparability	Outcome			
	Adequate definition of cases	Representativeness of cases	Selection of control subjects	Definition of control subjects	Control for important factor or additional factor[2]	Exposure assessment	Same method of ascertainment for all subjects	Non response rate	Total quality scores
Tavani, 1995 (460)	✸	✸	-	-	✸	-	✸	✸	5
Keramat, 2008 (456)	✸	✸	✸	✸	-	-	✸	-	5
Grgurevic, 2010 (461)	✸	✸	-	✸	✸✸	✸	✸	✸	8

[1] A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor.
[2] A maximum of 2 stars could be awarded for this item. Studies that controlled for calcium and vitamin D supplement use received one star, whereas studies that controlled for other important confounders (smoking, hormone replacement therapy, or body mass index) received an additional star.

Bridge Statement 4

Results from the systematic review and meta-analysis presented in Chapter 5 suggest that yogurt may be protective against hip fracture risk, but the overall quality of the evidence was very low. Based on the few RCTs included in the systematic review, FMP interventions significantly reduced markers of bone resorption (455, 462, 463). The observed benefits of FMPs on skeletal health outcomes require further clarification. Similarly, the underlying mechanism of the potential protective effect of FMP on CVD and related mortality has not been rigorously examined in experimental settings in humans (47, 478). There is considerable evidence showing that inflammation plays an important role in vascular calcification and bone remodeling. Taken together, modulation of inflammatory mediators may explain the protective effect of FMP consumption on vascular and bone health outcomes, but further investigational studies are warranted.

The Calcium Study, an RCT led by our research group, was designed to provide an evidence-based approach to answer the question regarding the impact of calcium supplementation on vascular and bone health in healthy postmenopausal women. The Calcium Study had a similar design to the pilot trial described in Chapter 3, with the addition of a control arm and the use of the calcium-focused FFQ as described in Chapter 4. The RCT examined the differential effect of supplemental versus dietary calcium on composite endpoints of arterial stiffness and arterial thickness, and found no detectable differences in carotid-femoral pulse wave velocity or cIMT at the end of the 12-month intervention compared to the control arm. However, a 12-month intervention might not be long enough to detect subclinical changes between the treatment arms. In contrast, changes in circulating biomarkers associated with CVD risk have been reported following short-term nutrition interventions (43, 45, 292, 479-483). Thus,

using the data from the Calcium Study, Chapter 6 investigated the effect of calcium, either from dietary sources alone or predominantly from supplemental sources, on selected inflammatory and bone biomarkers over 12 months as compared to the control group.

CHAPTER 6

Manuscript 4: Randomized Controlled Trial

Effect of calcium intake from dietary sources alone or combined with supplemental calcium on selected inflammatory and bone health biomarkers in healthy postmenopausal women: a 1-year randomized controlled trial

Angel M. Ong[1,2], Hope A. Weiler[2], Stella S. Daskalopoulou[1,4], David Goltzman[1,4] and Suzanne N. Morin[1,4]

Authors' Affiliation:

[1] School of Human Nutrition, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

[2] Research Institute of the McGill University Health Centre, 5252 de Maisonneuve Ouest, Montreal, Quebec, Canada, H4A 3S5

[3] Department of Medicine, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec, Canada, H3G 1Y6

Abstract

The benefits and risks of supplemental calcium require clarification. This study aimed to examine the short-term effect of calcium at the Recommended Dietary Allowance (RDA) level, either from dietary calcium alone or in combination with supplemental calcium on biomarkers implicated in vascular and bone health. Healthy postmenopausal women (*n*=121, BMI >19 <35 kg/m^2) were randomized to 1 of 3 groups for 12 months: CaSuppl (750 mg supplemental calcium + 450 mg dietary calcium + 800 IU vitamin D supplement [vitD] daily), CaDiet (1200 mg dietary calcium + 400 IU vitD daily), or Control (400 IU vitD daily). Baseline and 12-month measurements included plasma cytokines, serum high-sensitivity C-reactive protein (hsCRP), ionized calcium (iCa), 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), sclerostin, and the bone turnover markers (BTMs) bone-specific alkaline phosphatase and cross-linked C-telopeptide of type I collagen. Anthropometric measurements were also assessed. Linear mixed models were used to examine between and within-group differences over time. Linear regressions were performed to assess the association between different types of dairy products with biomarkers at baseline and at 12-months. One hundred and six (87.6%) participants completed the study. At 12-months, there were no between or within-group differences in hsCRP, interleukin-6, adipokines, iCa, PTH, or sclerostin. In contrast, BTMs decreased over time in both intervention groups, whereas 25(OH)D increased in all groups. BMI and percent body fat increased at 12-months in CaDiet only (*P*=0.04 and *P*=0.03, respectively). Overall dairy intake, total or by type, did not associate with inflammatory markers or BTMs. However, yogurt intake associated positively with 25(OH)D and serum sclerostin at baseline. In healthy postmenopausal women, after 12 months, calcium at the RDA level from either dietary or supplemental intakes did not affect inflammatory biomarkers but decreased markers of bone turnover. Whether intakes exceeding the RDA are implicated in vascular health remains uncertain.

6.1 Introduction

The current Recommended Dietary Allowance (RDA) for calcium is 1200 mg/day for women >50 years old to mitigate the rapid loss of bone mass at the time of menopause, and to counter the effect of aging on bone loss after the age of 70 years (370). The North American diet provides a variety of foods rich in calcium, including milk, milk products, and some non-dairy foods (3, 484). Yet, calcium intake from food sources among postmenopausal women is often below the RDA (estimated to average 800 mg/day in North America) and supplemental calcium is often recommended (3, 4, 485).

Higher calcium intakes from dietary or supplemental sources are associated with reduced rates of bone loss and fracture in older adults (367, 454, 486, 487). However, an increased risk of cardiovascular disease (CVD) and cardiovascular events associated with calcium supplementation have been reported. Increased rates of myocardial infarction (RR 2.12, 95% CI 1.01-4.47) were reported in healthy postmenopausal women who received 1 g/day of elemental calcium in a 5-year randomized clinical trial (RCT) compared to placebo (98). Similarly, meta-analyses from the same authors demonstrated an increased risk of myocardial infarction associated with calcium supplementation alone (HR 1.31, 95% CI 1.02-1.67) (8) or with co-supplementation of vitamin D (HR 1.21, 95% CI 1.01-1.44) (9). Other reports documented opposite or null results with doses of supplemental calcium that ranged from 0.5 g/day to 1.6 g/day (10-15). Total calcium intake from dietary and supplemental sources in these studies often exceeded the RDA and approached the Tolerable Upper Intake Level (UL, 2000 mg/day). Although rarely achieved from food sources alone, total calcium intake above the UL has the potential to cause hypercalcemia and promote calcification of vascular and soft tissues (370).

By contrast, higher dietary calcium intakes alone are generally associated with a lower

prevalence of CVD, and calcium intake from milk and milk products are mostly associated with cardio-protective benefits or no association with CVD risk (33, 34, 83, 85, 488, 489). However, findings from a large cohort of Swedish women suggest that for every 200 g of milk consumed, there was an increased risk of all-cause mortality (adjusted HR 1.15, 95% CI 1.13-1.17) and cardiovascular mortality (adjusted HR 1.15, 95% CI 1.12-1.19) (84). In contrast, fermented milk product (FMP) intake, such as yogurt and cheese, was associated with lower rates of mortality (84). This suggests that the complex nutrient matrices of different dairy foods may have varying properties, such as pro- and anti-inflammatory effects on health (40). Findings of a recent meta-analysis of 29 prospective cohort studies did not show an association between total dairy nor milk consumption with total mortality, coronary artery disease or CVD (41). Nonetheless, FMP consumption was associated with a modestly lower risk of total mortality and CVD (41). Thus far, emerging evidence suggests that FMP may be associated with lower CVD risk compared to non-fermented milk (46).

Although the mechanism by which supplemental calcium could increase cardiovascular risks remains speculative, the development of coronary artery disease has been suggested (101, 490). Inflammation may be involved as it plays a central role in the pathogenesis of atherosclerosis and aortic calcification (108-110, 491). Elevated circulating inflammatory markers have been shown to be associated with coronary arterial calcification, CVD and mortality (113-116). Furthermore, biomarkers involved in bone mineralization are also associated with CVD (241, 256-258, 492-494). However, important knowledge gaps remain concerning the underlying mechanisms through which supplemental calcium may exert adverse effects on cardiovascular health due to a lack of RCT designed to address this question.

To gain understanding into the underlying associations between calcium and vascular risk,

we used the biochemical data obtained from a 1-year RCT designed to estimate the effect of dietary calcium as compared to supplemental calcium on surrogate markers of vascular health in healthy postmenopausal women. Our objective was to examine whether calcium intake through different forms (supplemental versus dietary) has differential effects on selected inflammatory and bone biomarkers in healthy postmenopausal women. Secondary objectives were to assess the effects of supplemental and dietary calcium on body composition. The third objective was to examine the relationship between individual milk products and the selected inflammatory and bone biomarkers.

6.2 Methods

Participants

Healthy postmenopausal women (2 or more years since last menstrual period) ≥50 years of age without any known chronic disease were recruited through posters and local newspaper advertisements in the greater Montreal (Quebec, Canada) area from January 2014 to November 2017. Exclusion criteria were established to avoid including women with clinical or subclinical vascular impairment due to cardiovascular risk factors or CVD. Specifically, women who were smokers within the last 5 years were excluded, as were those who had a BMI <19 or >35 kg/m^2, history of diabetes, pre-eclampsia, hypertension, atrial fibrillation or atherosclerosis. Participants who had used hormonal therapy (excluding vaginal preparations) in the last 3 years, medications to treat hypertension or hypercholesterolemia, or medications known to affect bone metabolism within the past 12 months were excluded. Those with a 10-year absolute risk of major osteoporotic fractures >20 %, computed using FRAX^{TM} without bone mineral density, were also excluded (321). During screening, if potential participants were taking nutritional supplements that contained calcium and/or vitamin D, they were asked to stop the supplement intake for 2

months prior to participation (washout period).

Study design

This was a 12-month RCT (*ClinicalTrials.gov* ID: NCT01731340). Participants were initially randomly allocated to 1 of 3 parallel groups by permuted block size of 9 (1:1:1) on the day of the baseline visit. Due to strict eligibility criteria, recruitment rates were low, and thus the randomization ratio was subsequently (October 27, 2015) changed to 4:4:1 to increase enrollment to the intervention arms. As of November 22, 2016, randomization to the control group was discontinued, and newly enrolled participants were randomized to either one of the two intervention groups by permuted block size of 6 (3:3:0). The protocol was amended accordingly to reflect both changes in the allocation ratio.

A web-based randomization algorithm was used to generate permuted block randomization and the allocation sequence was concealed. Participants were informed of their group assignment in person at the end of their baseline visit by the research dietitian, who was aware of the result of the randomization. Outcome assessors were blinded throughout the study and participants were asked to not reveal their treatment allocation to research staff at follow-up visits.

The interventions were as follows: Calcium Supplement group (CaSuppl): 750 mg calcium citrate supplement (Ci-Cal) + 450 mg from food sources + 800 IU vitamin D supplement (Euro-D 800) daily; or Dietary Calcium group (CaDiet): 1200 mg of calcium from food sources + 400 IU vitamin D supplement (Euro-D 400) daily; or Control group: *ad libitum* diet + 400 IU vitamin D supplement daily for 12 months. All supplements were purchased from Euro-Pharm International Canada Inc. (Montreal, Quebec). Interventions for CaSuppl and CaDiet were designed to meet the RDA for calcium at 1200 mg/day and vitamin D at 600-800 IU/day.

CaSuppl participants were instructed to limit their intake of calcium-rich foods, particularly milk, milk products and alternative calcium-rich foods, to one small portion per day (providing approximately 150 mg/portion). Participants randomized to CaDiet were instructed to increase their intake of calcium rich foods to 3 portions per day (providing approximately 300 mg/portion). While few foods naturally contain vitamin D, the CaDiet intervention supplies approximately 400 IU of dietary vitamin D from fluid milk and plant-based beverages enriched with calcium. To balance vitamin D intakes across groups, a higher dose of vitamin D supplement was provided to CaSuppl participants to compensate for a lower intake from dietary sources. Foods were considered rich in calcium if they provide ≥100 mg per recommended serving sizes as per the 2007-2019 Canadian Food Guide (63): fluid milk (300 mg per 250 ml), yogurt (189-257 mg per 175 g), cheese (100-426 mg per 50 g), plant-based beverages enriched with calcium (300 mg per 250 ml), canned fish with bones (179-212 mg per 75 g) and tofu prepared with calcium (302-525 mg per 100 g). An additional 300 mg of dietary calcium from other foods, such as vegetables and grains (322), were taken into consideration in the design of the study interventions to meet the RDA.

Assessments

Anthropometric measurements

All assessments were performed in the fasted state following a 12-hour overnight fast. Height, weight, percent body fat, and waist and hip circumferences were measured at each visit using standard practices. Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 242, Seca, Hamburg, Germany). Weight and percent body fat were measured using bioelectrical impedance analysis (Tanita TBF-310; Tanita Corporation, Arlington Heights, IL, USA) to the nearest 0.2 kg and 0.1%, respectively, in light clothing

without shoes. Waist circumference was measured at the end of a normal expiration at the superior border of the iliac crest to the nearest 0.5 cm using a nonflexible body measuring tape over the skin (495, 496). Hip circumference was measured to the nearest 0.1 cm at the level of the symphysis pubis and the greatest gluteal protuberance (331).

Demographic characteristics, physical activity and health survey

Medical history, medication and nutritional supplement use were reviewed at the baseline visit. The long-form International Physical Activity Questionnaire (IPAQ) was administered at baseline, 6-months and 12-months to estimate physical activity level in the last 7 days (325, 326). General health status, use of new medications and nutritional supplements were also surveyed during monthly telephone follow-ups.

Dietary assessment and compliance

We have previously developed and validated a calcium-focused 30-day semi-quantitative food frequency questionnaire (FFQ) (497). The 51-item FFQ had a high inter-rater agreement (intra-class correlation coefficient 0.93, 95% CI 0.82-0.97) and a systematic bias of underestimating dietary calcium intake by approximately 99 ± 295 mg/day. The FFQ was administered by the research dietitian at baseline, 6-months and 12-months to determine usual dietary calcium and vitamin D intake. Participants were asked about the frequency of consumption (never or times per month/week/day, as appropriate) of the items included in the FFQ during the previous month (e.g., "How frequently did you have yogurt during the past month?") followed by a question about the usual portion size consumed to enhance reporting accuracy (e.g., "When you had yogurt during the past month, how much did you usually have each time?"). Reported frequencies and quantities were entered in an automated Microsoft Excel 2010 spreadsheet (Redmond, WA, USA) and the average daily dietary calcium intake for each

item and the daily total dietary calcium intake were automatically calculated. The Canadian Nutrient File 2010b database was used to calculate the average values of the calcium content of each item on the FFQ (484).

Multiple-pass 24-hour recalls were administered at each on-site visit and at each monthly telephone follow-up (329). Participants were asked to recall all foods and beverages they consumed during the day before each visit and before each scheduled telephone follow-up. During the initial visit, participants received detailed instructions and a portion size guide (www.fns.usda.gov) adapted to the Canadian context with examples of visual cues using common household items to help them estimate quantities of food during telephone interviews (389). Intake data from each 24-hour recall were entered into the Nutritionist Pro software version 5.2.0 (Axxya Systems, Stafford, TX, USA) to calculate intakes of dietary calcium, vitamin D and energy using the Canadian Nutrient File 2010b database maintained by Health Canada (484). Nutrient content of foods that were not available in the database were determined by verification of food labels or estimated by the study dietitian. Nutrition data were audited for transcription accuracy and assumption consistency.

Dietary calcium intake data obtained from the monthly follow-ups were used to monitor adherence to the dietary interventions. Participants received feedback from the research dietitian at the end of each follow-up and received further guidance as needed. Supplementation compliance was assessed by participant-reported tablet count during monthly follow-up calls and by verification of reported tablet count upon return of supplement bottles at 6- and 12-month visits. Compliance with the trial interventions was defined as an average total calcium intake within 20% of target, as per intervention assignment (e.g., 1200 ± 240 mg in CaDiet), and use of calcium supplements ≥80%. Health status and adverse events, including cardiovascular,

musculoskeletal, renal and gastrointestinal adverse events were also monitored at each monthly follow-up.

Biomarkers

Fasting blood samples were collected between 07:00 and 11:00 hours at baseline and at the 12-month final visits to minimize potential diurnal variation of the biomarkers. Ionized calcium was analyzed within 30 minutes of sampling at the Division of Biochemistry at the Montreal General Hospital (MGH) using the ABL 800 blood gas analyzer (Radiometer Canada, London, Ontario, Canada), with an inter-assay percent coefficient of variance (CV) of 5%. The remaining blood samples were separated into plasma and serum fractions and stored at −80°C until further analysis. Serum hsCRP was measured using Beckman AU5800 (Beckman Coulter Diagnostics, Brea, CA) at the MGH with an inter-assay CV of 11.5%. Serum cross-linked C-telopeptide of type I collagen (CTX) was measured by electrochemiluminescence immunoassay (cobas e-411, Roche Diagnotics, Mannheim, Germany) with an inter-assay CV of 8%. Serum 25(OH)D, intact 1-84 PTH and bone-specific alkaline phosphatase (BSAP) concentrations were measured at McGill University in the School of Human Nutrition. Serum 25(OH)D concentrations were measured using the LIAISON Total 25(OH)D assay (DiaSorin Inc, Stillwater, MN, USA). The intra- and inter-assay CVs were <5% and the accuracy was 91-93% for the National Institutes of Standards and Technology Standard Reference Material (NIST) 972a Level 2 Vitamin D metabolites in frozen human serum. This laboratory participates in the DEQAS (Vitamin D External Quality Assessment scheme) program and obtained a certificate of Proficiency for 2015-2016, which reflects that 75% or more of the reported results fell within 25% of target values. PTH concentrations were measured using the LIAISON 1-84 PTH assay. The intra-assay and inter-assay CVs were <8.0% and the accuracy was between 93-113% for

DiaSorin 1-84 PTH Controls. BSAP concentrations were measured using the LIAISON BAP Ostase assay. The intra-assay and inter-assay CVs were <8.5% and the accuracy was between 81-100% for DiaSorin BAP Ostase Controls. Serum sclerostin was measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Human Sclerostin HS EIA kit, TECOmedical group, Sissach, Switzerland) with an inter-assay CV of 7.4% and an accuracy of 95-111%. Plasma IL-6, total adiponectin, high molecular weight (HMW) adiponectin and leptin were measured using ELISA (R&D Systems, Minneapolis, MN, USA). The inter-assay CVs and accuracy were 7.3% and 80-106% for IL-6, 6.2% and 69-107% for total adiponectin, 4.6% and 78-87% for HMW adiponectin, and 0.5% and 84-93% for leptin. Controls were within the ranges as per the manufacturer's specifications for each ELISA assay and assay accuracy were calculated using the mid-range of manufacturer specifications for controls for all biomarkers with the exception of 25(OH)D.

Ethics

The present trial was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethics approval was granted by the McGill University Health Centre Research Ethics Board (GEN-11-231). Written informed consent was obtained from all participants.

Sample size calculation

This paper is a secondary analysis of the RCT and the sample size was based on the main outcome of the RCT (498). With an anticipated loss to follow-up of 20% based on previous calcium studies (11, 98, 316, 344, 366), a sample size of 60 participants per group was calculated based on published data on the effect of dairy food intake on arterial stiffness to detect a 10% change in cfPWV with 80% power after a 12-month intervention. (74). No specific power calculation was performed for the present analysis.

Statistical analysis

Statistical analyses were performed based on the intention-to-treat principle which included data from all participants. In order to examine the robustness of the results to assumptions made in the primary analysis, complete case analyses were also performed for primary and secondary outcomes (499).

Data were audited and tested for normality using the Shapiro-Wilk test (390). Non-normally distributed data were log-transformed. If log-transformed data remained non-normally distributed, then a non-parametric test was performed. Summary statistics were computed for baseline characteristics, presented as mean values and standard deviations for normally distributed continuous variables, medians and interquartile ranges for non-normally distributed variables, or as counts and percentages for categorical variables. The $\chi 2$ test was used to assess for differences in proportions. One-way analysis of variance was used to compare baseline differences between groups. Linear mixed models were used to analyze between-group differences over time in biomarkers and anthropometric measurements with Tukey *post-hoc* tests. Fixed effects included group, time-point and their interaction, whereas random effects included the subject variable to account for variation that is due to individual differences (500). Associations between total milk products, milk, yogurt and cheese intake, and selected inflammatory and bone biomarkers at baseline and 12-months were assessed using separate linear regression models. Multiple covariates and potential confounders were considered in the linear models: age, BMI, 25(OH)D, PTH, physical activity, and the assigned treatment group (501-507). All baseline linear models were adjusted for age and BMI and other relevant covariates that may influence the dependent variable based on previously published evidence (508-511). Models for HMW adiponectin, total adiponectin, and leptin were further adjusted for

25(OH)D, and physical activity. Models for BSAP, CTX and sclerostin were further adjusted for 25(OH)D, physical activity, and PTH. Models at 12-months were further adjusted for the assigned treatment arm. Significance was set at $P<0.05$. All statistical analyses were performed using the R statistical program version 3.6.1 and additional R packages 'lme4' (512) and 'lmerTest' (513).

6.3 Results

Demographic characteristics and adherence

One hundred and twenty-one healthy postmenopausal women were randomized between March 2014 and November 2017 (**Figure 6.1**). Mean age was 60.4 (SD 6.0) years and the average BMI and waist circumference were 25.7 (SD 4.0) kg/m^2 and 90.5 (SD 10.4) cm, respectively. Fifteen participants withdrew from the study of whom 9 were from CaSuppl (19%), 4 from CaDiet (8%) and 2 from the Control arm (8%).

No differences in baseline characteristics were observed among the 3 groups in terms of age, anthropometric measurements, dietary calcium intake, vitamin D intake, alcohol consumption, smoking history, and use of calcium and/or vitamin supplements prior to trial entry, except for physical activity (**Table 6.1**). Median physical activity level was lower in CaDiet (median 38.2 (IQR 20.5-57-5) MET-hr/week) compared to Control (median 60.4 [IQR 35.8-88.2] MET-hr/week), P=0.0245). There was no seasonal effect on the recruitment rate among groups, and 25(OH)D concentrations (mean 65.8 [SD 20.3] nmol/L) were balanced among groups at baseline.

Overall, 106 participants completed all the telephone follow-ups and on-site visits. CaDiet had a higher average energy intake compared to CaSuppl at 1-6 months (1839 [SD 326] kcal vs. 1538 [SD 297] kcal, P=0.009) and at 7-12 months (1816 [SD 340] kcal vs. 1514 [SD

315] kcal, *P*=0.008). Dietary data derived from the multiple-pass 24-hour recalls indicated that 36 (95%) CaSuppl participants were within 20% of their target total calcium intake over the 12-month period that ranged between 387-660 mg/day (median 526 [IQR 488-586] mg/day). In CaDiet, 42 (95%) adhered to the CaDiet protocol with an average dietary calcium intake that ranged between 992-1416 mg/day (median 1218 [IQR 1122-1292] mg/day). Adherence rate for calcium supplement use was 97.4%, and ranged from 97.4-100% for vitamin D supplement use across the 3 groups. Calcium and vitamin D intakes by source are presented in **Table 6.2**. Participants from each group reported adverse events including constipation (*n*=24), joint pain (*n*=60), nausea (*n*=26), abdominal pain (*n*=27), muscle pain (*n*=60), palpitations (*n*=3). Three women reported having sustained a fracture during the study. No cardiovascular adverse events were reported.

Selected inflammatory and bone biomarkers

At baseline, there were no differences in hsCRP, IL-6, total adiponectin, HMW adiponectin, or leptin among groups (**Table 6.3**). At 12-months, no between-group differences or significant change over time were observed. PTH concentrations were all within the normal clinical range but slightly higher in Control compared to CaSuppl (*P*=0.0106) and CaDiet (*P*=0.0107) at baseline (**Table 6.3**). Ionized calcium was higher in Control compared to CaDiet, though serum ionized calcium levels were well within the normal clinical range in all participants. At 12-months, no between-group differences were observed. A decrease in BSAP at 12-months was only observed in CaSuppl (*P*=0.005) whereas a decrease in CTX was observed in both CaSuppl (*P*=0.003) and CaDiet (*P*=0.002). No change in PTH over time was observed, and 25(OH)D increased at 12-months in all three groups (*P*<0.0001). There were no between-group differences at any time point or within-group differences over time in serum sclerostin levels.

We also explored models with age, BMI, and physical activity as covariates, but these variables were not included in the final regression models as they did not alter the results after adding group, time-point, and their interaction as fixed effects. Similar results were obtained when the analysis was restricted to the complete cases.

Body composition

No differences in BMI, body weight, percent body fat, waist circumference or hip circumference were seen among groups at baseline or at 12-months (**Table 6.4**). In CaDiet, there was an increase in BMI at 12-months from baseline (26.1 [SD 4.4] kg/m^2 *vs.* 25.8 [SD 4.2] kg/m^2, *P*=0.04), body weight (68.0 [SD 11.5] kg *vs.* 66.7 [SD 10.8] kg, *P*=0.01), and percent body fat (35.4% [SD 6.3] *vs*. 34.5% [SD 6.2], *P*=0.03). Similar results were obtained when the analysis was restricted to complete cases.

Association of milk product intake with selected inflammatory and bone biomarkers

At baseline, cheese consumption (median 4.2 [IQR 2.2-6.6] servings/week) was higher than milk (median 3.4 [IQR 0.4-6.1] servings/week; *P*=0.006) and yogurt consumption (median 3.3 [IQR 1.1-5.5] servings/week; *P*= 0.01). At 12-months, the overall intake of milk (median 2.0 [IQR 0.6-6.0]), yogurt (median 2.3 [IQR 0.5-5.0]) and cheese (median 3.2 [IQR 1.1-5.2]) were not different (*P*=0.40). **Figure 6.2** depicts the distribution of intake of total milk products, milk, yogurt and cheese by group at baseline and at 12 months. Total milk products, milk, yogurt, and cheese intakes were not associated with inflammatory biomarkers (**Table 6.5**), except with log-transformed total adiponectin (ß=0.017, *P*=0.01) at baseline. However, the results were not significant in the adjusted model. There was a positive association between total milk product in the unadjusted model (ß=0.73, *P*=0.004), yogurt (ß=1.36, *P*=0.01), and cheese (ß=01.23, *P*=0.02) intake with serum 25(OH)D at baseline, and these associations remained significant in the

adjusted models for total milk product and yogurt intake. Total milk product was positively associated with sclerostin (ß=0.2, *P*=0.03) at baseline. Yogurt consumption was also positively associated with serum sclerostin at baseline in both unadjusted (ß=0.6, *P*=0.02) and adjusted models (ß=0.6, *P*=0.01). No significant relationships were observed at 12-months overall. We did not perform analyses to examine the association between alternative sources of calcium-rich foods (eg. plant-based beverages fortified with calcium, tofu prepared with calcium) with the selected biomarkers due to the overall very low consumption of these foods by participants in our study.

6.4 Discussion

We investigated the impact of calcium from dietary and supplemental sources at the RDA level on selected inflammatory and bone biomarkers in healthy postmenopausal women. The RDA level was chosen to conform to current dietary guidelines, in contrast to other studies in which calcium intake from elemental calcium and dietary intake combined approached or even exceeded the UL for calcium (11, 12, 98, 316, 344, 366, 514-522). In our study, calcium from dietary sources alone, or in combination with 750 mg of supplemental calcium, did not affect serum hsCRP, or plasma IL-6, adiponectin or leptin. The main effects observed were an increase in serum 25(OH)D across all trial arms and a decrease in bone turnover markers in the intervention arms. The increase in serum 25(OH)D can be explained by the high adherence to the study supplements. BSAP concentrations decreased in CaSuppl, whereas CTX concentrations decreased in both intervention arms and no change was observed in Control. The observed decrease in bone turnover markers is consistent with other interventional studies of dietary or supplemental calcium intervention in postmenopausal women (486, 523-525). Our findings suggest that the effect of dietary and supplemental calcium at the RDA level have similar effects

on bone turnover in vitamin D sufficient healthy postmenopausal women, and may signify a beneficial skeletal action as bone turnover may be increased in postmenopausal women and contribute to the development of osteoporosis (526).

We did not find significant differences between arms at baseline or over time in IL-6, hsCRP, adiponectin or leptin. Furthermore, we did not observe any significant associations between individual milk product intake and the selected inflammatory markers. IL-6 has been implicated in adverse cardiovascular health during the initial stages of atherosclerosis development and elevated levels may directly reflect vascular inflammation and endothelial injury (140, 527). On the other hand, elevated leptin concentrations are generally associated with the pathogenesis of hypertension and arterial stiffness (151, 528). Few trials have investigated the effect of calcium supplementation (≤1 year duration) on inflammatory markers and found no effect on circulating inflammatory biomarkers (291-294). Our findings are also consistent with RCTs of short-term milk or supplemental interventions in postmenopausal women (43, 293, 294, 529, 530). In a small 12-week non-randomized trials (*n*=39), daily supplementation with 1000 mg calcium and 800 IU vitamin D had no effect on CRP or IL-6 and other circulating cytokines in healthy postmenopausal women (529). Similarly, an RCT in healthy postmenopausal women (*n*=116) reported no change in CRP level after 1 year of daily supplementation with 1000 mg calcium (294). In women with intermediate cardiovascular risk (*n*=117), daily intake of 500 mL of semi-skimmed milk did not affect the hsCRP levels after a 12-month intervention (531). FMPs may exhibit anti-inflammatory effects resulting in decreased levels of pro-inflammatory cytokines (300-302), but we were unable to examine these inferred effects due to the relatively low consumption. Nonetheless, our findings suggest that dietary calcium or supplemental calcium at the level of the RDA does not affect inflammatory markers in healthy postmenopausal

women.

An increase in serum ionized calcium concentration has been speculated as one of the key mechanisms accelerating vascular calcification by calcium supplementation, via a loss of inhibition of mineralization due to increased complexing of ionized calcium with pyrophosphate and reduced inhibition of arteriosclerotic signaling in vascular smooth muscle cells due to decreased PTH levels (101). Higher serum calcium concentrations have been associated with carotid artery plaque thickness (347) and increased risk of myocardial infarction and stroke (348) in older adults. In our study, serum ionized calcium concentrations were slighted decreased at 12-months in CaSuppl and Control, but the concentrations remained within normal clinical ranges and no differences between or within the intervention arms were observed. We also observed no differences in serum PTH or sclerostin concentrations between or within arms over time, and both biomarkers play a crucial role in bone metabolism and mineralization. Findings from trials of supplemental calcium also do not support the speculated mechanism (13, 532). A 6-month randomized feeding trial with high calcium diets from either dairy or calcium carbonate to reflect intake at the UL level in Ossabaw miniature swine could not demonstrate worsened indices of cardiovascular function or arterial calcification compared to the control group fed an atherogenic diet (532). Findings from an ancillary study found no increase in carotid intima media thickness following 3 years of calcium carbonate supplementation at 1.2 g/day compared to placebo in elderly women >70 years (13). This body of evidence indicates that calcium, regardless of the source, does not promote vascular calcification.

An inverse association between calcium intake and body weight have been reported in observational studies (533-535), but the relationship between dairy product intake and body composition is supported by mixed findings (536). Calcium supplementation trials have not

demonstrated a reduction in body weight or fat mass in postmenopausal women (537-540). We observed an increase in BMI, body weight and percent body fat in CaDiet at 12-months. The observed weight gain of 1.3 kg (1.9% gain) over 1 year was not clinically significant (541). In comparison to previous studies that demonstrated body weight reduction as a result of dairy interventions, participants in our study did not follow a hypocaloric regimen (542). In our study, no dietary advice other than ways to adjust their intake of calcium-rich foods was provided. As a result, participants in CaDiet likely added calcium-rich foods into their diet instead of substituting for other foods given that physical activity did not change over time. However, we were unable to capture a statistically significant increase in energy over time to confirm this assumption because we only performed one single 24-hour recall at baseline. Nonetheless, we did not observe a significant change in inflammatory cytokines at the end of the study that would have been expected with clinically significant weight gain (543, 544). Moreover, similar results were reported in an RCT that assessed the impact of increasing fluid milk intake (skim or 1% milk fat) to 3 servings/day, without other dietary advice, on energy and nutrient intake, and body weight and cardiovascular risk factors in healthy adults 55 to 85 years of age (545). The intervention group gained 0.6 kg more than the control group that maintained a usual diet with <1.5 dairy servings/day, but blood pressure decreased over time in both groups and plasma lipid levels were unchanged or remained within the normal range.

The consumption of milk and milk products in the entire study sample was relatively low at baseline (~1 serving/day). Total and individual milk products did not associate with inflammatory markers or bone health markers, with the exception of serum 25(OH)D and sclerostin. It was interesting to observe a positive association between total milk product and yogurt intake, but not fluid milk, with 25(OH)D concentrations at baseline. There is a mandatory

fortification of fluid milk, but not for other dairy products, with vitamin D in Canada. We also found a weak albeit positive association between yogurt consumption with serum sclerostin at baseline. Sclerostin reduces bone formation and stimulates bone resorption (268, 269), and higher sclerostin levels have been associated with lower bone mineral density, increased fracture risk (277), as well as increased cardiovascular risks (546, 547), though the evidence for bone health outcomes is mixed. This may be due to the limited number prospective studies, lack of repeated measurements of sclerostin, use of different assays, and heterogeneity in population groups studied. On the other hand, studies have shown the benefits of yogurt on bone health (415, 471, 548) and vascular health (85, 478, 549). Thus far, studies have shown a lack of association or lack of an effect of dairy intake and supplemental calcium with sclerostin levels in postmenopausal women (275, 550, 551), though there is currently a paucity of published research exploring this relationship. The observed positive association between yogurt intake and serum sclerostin in this study should be interpreted with caution given the cross-sectional nature of the analysis and the relatively low intakes of dairy foods overall at baseline. There is insufficient evidence to indicate whether interventions of calcium in the form of supplement or the diet have an effect on sclerostin levels in postmenopausal women and this warrants further research.

Our work has several strengths, including the rigorous design. The early education intervention by the research dietitian 1 week following randomization and subsequently on a monthly basis resulted in high adherence. Specifically, the monthly follow-ups ensured understanding and reinforced compliance to the study protocol as evidenced by the high adherence in all arms. The use of our validated calcium-focused FFQ to complement dietary intake data collected with the 24-hour recall is also a strength of this study. We performed

sensitivity analyses to assess the robustness of the results from the intention-to-treat analysis by assessing bias that could arise from difference between women who did and did not complete the trial (499).

Although our trial was limited by its small sample size and short duration of the intervention, we observed changes in bone turnover markers following a 12-month intervention in a highly adherent group of postmenopausal women. Our trial was unable to fully compare the effect of supplemental and dietary calcium given that dietary calcium is integrated in all study arms and the two sources of calcium are not mutually exclusive because a typical diet provides approximately 300 mg calcium from foods like vegetables and grains (322). However, our trial interventions reflected a more realistic and practical approach to meet the RDA from dietary and supplemental sources of calcium. Lastly, our results cannot be generalized to groups other than healthy postmenopausal women. We elected to recruit healthy postmenopausal women as in our main trial we sought to understand the mechanism underpinning the effect of calcium supplementation versus dietary calcium on vascular health.

In conclusion, calcium intake at the level of the RDA from either diet alone or combined with supplemental sources had no detectable effect on selected inflammatory and bone biomarkers in healthy postmenopausal women. This study adds to the body of evidence supporting the hypothesis that calcium intakes that meet current dietary recommendations, regardless of the source, do not pose a cardiovascular risk in healthy postmenopausal women in the short term. Whether exceeding the recommended intakes is implicated in vascular health, especially in women with predisposed vascular risks, warrants further research.

Table 6.1 Participant characteristics at baseline

Characteristics	CaSuppl (n = 47)	CaDiet (n = 48)	Control (n = 26)
Age (y)	60.5 ± 6.0	60.5 ± 6.2	59.8 ± 5.7
Menopause age (y)	51.0 (48.5, 53.0)	50.0 (46.0, 53.0)	51.0 (50.0, 53.0)
Ethnicity, White (n, %)*	43 (91%)	43 (90%)	25 (96%)
BMI (kg/m^2)	25.9 (23.7, 27.2)	24.9 (22.8, 28.1)	23.3 (21.3, 28.5)
Waist circumference (cm)	90.9 ± 9.4	90.8 ± 10.6	89.3 ± 11.8
Hip circumference	104.0 (101.1, 110.0)	101.3 (97.0, 107.0)	100.0 (96.0, 106.8)
Waist-to-hip ratio	0.86 ± 0.06	0.88 ± 0.06	0.88 ± 0.07
Percent body fat (%)	36.3 ± 5.8	34.5 ± 6.2	33.6 ± 7.3
Physical activity (MET-hr/week)	40.0 (27.2, 69.1)[a,b]	38.2 (20.5, 57.5)[a]	60.4 (35.8, 88.2)[b]
Ex-smoker (n, %)	22 (46%)	23 (48%)	16 (62%)
Calcium (mg/d)†	839 (620, 1037)	828 (635, 1079)	789 (506, 994)
Vitamin D (µg/d)†	6.1 (4.5, 7.8)	5.7 (3.8, 7.8)	4.9 (3.5, 6.5)
Alcohol consumption (n, %)			
None	5 (11%)	4 (8%)	2 (8%)
< 9 drinks/week	34 (72%)	39 (81%)	21 (81%)
≥ 9 drinks/week	8 (17%)	5 (10%)	3 (12%)

Data are presented as mean ± SD or median (IQR) or *n* (percentage).
BMI, body mass index; CaSuppl, Supplemental Calcium group; CaDiet, Dietary Calcium group.
† Calcium and vitamin D intakes were assessed from the calcium-focused food frequency questionnaire to reflect usual intake of the past month.
* Non-white includes Black (n = 4), Hispanic (n = 6) and Asian (n = 0).
[a,b] Different superscripts denote significant differences between groups (P=0.0245).

Table 6.2 Intake of calcium and vitamin D from dietary and supplemental sources by group over the 12-month study (per protocol)[1]

Sources	CaSuppl (*n*=36)		CaDiet (*n*=42)		Control (*n*=24)	
	Calcium (mg/d)	Vitamin D (IU/d)	Calcium (mg/d)	Vitamin D (IU/d)	Calcium (mg/d)	Vitamin D (IU/d)
Diet[a]	398 (316, 474)	147 (114, 187)	1039 (948, 1166)	281 (230, 339)	673 (511, 901)	176 (133, 279)
Supplements[b]	725 (708, 742)	790 (779, 797)	0	390 (380, 398)	0	395 (388, 399)
Total[c]	1123 (1056, 1188)	930 (899, 969)	1039 (948, 1166)	673 (628, 720)	673 (511, 901)	568 (529, 662)

Data are presented as median (IQR).

[1] Calcium and vitamin D intakes of participants who completed the study are presented (*n*=102) and excluded data from participants who did not adhere to the study interventions. One CaSuppl participant who had <80% adherence to study supplements and another CaSuppl participant who exceeded the assigned calcium target of 450 mg/day (median 575 [IQR 452-864] mg/day) were excluded. Two women in CaDiet who had an average calcium intake >20% of their assigned target over the study period were also excluded (median 1423 [IQR 1380-1565] mg/day; median 1471 [IQR 1427-1591] mg/day).

[a] Dietary data were derived from the calcium-focused semi-quantitative food frequency administered at 6 months and 12 months.

[b] Amounts of each nutrient derived from study supplements were calculated based on calculated adherence.

[c] Total intake of each nutrient was the sum of the diet and supplements.

Table 6.3 Differences among groups for inflammatory and bone health biomarkers at baseline and at 12 months in mixed models

	CaSuppl		CaDiet		Control	
	Baseline (n = 47)	12-month (n = 38)	Baseline (n = 48)	12-month (n = 44)	Baseline (n = 26)	12-month (n = 24)
Vascular biomarkers						
hsCRP (mg/L)	1.00 (0.55, 2.15)	1.00 (0.52, 1.80)	1.35 (0.47, 2.65)	1.00 (0.50, 2.40)	1.00 (0.62, 2.08)	1.20 (0.50, 2.80)
IL-6 (pg/mL)	1.98 (1.57, 2.42)	1.92 (1.57, 2.29)	1.71 (1.57, 2.67)	2.20 (1.57, 2.87)	1.83 (1.56, 2.37)	2.16 (1.57, 2.62)
Total adiponectin (µg/mL)	10.8 (7.5, 13.4)	11.1 (8.2, 14.4)	9.1 (6.1, 13.1)	8.5 (6.4, 12.8)	13.0 (7.6, 16.3)	12.8 (7.3, 16.2)
HMW adiponectin (µg/mL)	8.2 (5.8, 11.0)	7.6 (6.4, 10.0)	6.4 (4.6, 8.6)	5.9 (5.1, 10.1)	7.8 (5.5, 9.9)	7.1 (6.2, 10.4)
Leptin (ng/mL)	17.6 (9.9, 25.3)	20.9 (11.7, 29.0)	18.2 (12.1, 27.0)	18.1 (11.4, 33.4)	11.2 (6.2, 31.7)	12.2 (7.0, 35.6)
Bone biomarkers						
iCa (mmol/L)	1.24 ± 0.05[a,b]	1.20 ± 0.05[*]	1.22 ± 0.05[a]	1.20 ± 0.05	1.26 ± 0.03[b]	1.23 ± 0.05[*]
PTH (pmol/L)	2.6 (2.3, 3.2)[a]	2.4 (2.1, 2.8)	2.9 (2.2, 3.2)[a]	2.7 (2.1, 3.2)	3.2 (2.8, 4.2)[b]	3.0 (2.6, 3.8)
25(OH)D (nmol/L)	67.9 ± 20.5	80.1 ± 16.7[*]	65.4 ± 19.1	73.2 ± 14.9[*]	62.6 ± 22.3	71.7 ± 19.0[*]
Phosphate (mmol/L)	1.21 ± 0.10	1.18 ± 0.12	1.20 ± 0.12	1.19 ± 0.12	1.18 ± 0.09	1.18 ± 0.14
BSAP (µg/L)	14.6 (12.2, 17.4)	12.1 (9.9, 13.7)[*]	13.4 (10.5, 16.1)	12.8 (10.4, 15.0)	12.7 (11.3, 16.3)	12.4 (10.5, 15.5)
CTX (µg/L)	0.58 ± 0.17	0.48 ± 0.18[*]	0.58 ± 0.21	0.49 ± 0.14[*]	0.53 ± 0.15	0.50 ± 0.14
Sclerostin (pmol/L)	29.9 ± 8.8	30.8 ± 8.8	31.7 ± 8.8	30.8 ± 8.8	32.1 ± 9.7	32.1 ± 10.6

Data are unadjusted mean ± SD or median (IQR).
25(OH)D, 25-hydroxyvitamin D; BSAP, bone specific alkaline phosphatase; CaSuppl, Supplemental Calcium group; CTX, cross-linked carboxyl-terminal peptide; CaDiet, Dietary Calcium group; HMW adiponectin, high molecular weight adiponectin; hsCRP, high-sensitivity C-reactive protein; iCa, ionized calcium; IL-6, interleukin-6; PTH, parathyroid hormone.
[a,b] Different superscripts denote significant differences at baseline compared to Control (CaSuppl: PTH (P=0.0106); CaDiet: PTH (P= 0.0107), iCa (P=0.0042)).
[*] Denotes significant within group differences at 12-months from baseline (CaSuppl: BSAP (P=0.005), CTX (P =0.003), 25(OH)D (P<0.0001), iCa (P=0.0006); CaDiet: CTX (P =0.002), 25(OH)D (P<0.0001); Control: 25(OH)D (P<0.0001), iCa (P=0.04)).

Table 6.4 Differences among groups for anthropometric measurements at baseline and at 12 months in mixed models

	CaSuppl		CaDiet		Control	
	Baseline (n = 47)	**12-month (n = 38)**	**Baseline (n = 48)**	**12-month (n = 44)**	**Baseline (n = 26)**	**12-month (n = 24)**
Anthropometry						
BMI (kg/m^2)	26.0 ± 3.4	25.8 ± 3.8	25.8 ± 4.2	26.1 ± 4.4*	25.1 ±4.4	25.7 ± 4.7
Weight (kg)	68.3 ± 9.9	68.7 ± 11.4	66.7 ± 10.8	68.0 ± 11.5*	65.3 ± 11.5	66.9 ± 12.1
Waist circumference (cm)	90.9 ± 9.4	90.9 ± 11.1	90.8 ± 10.6	91.9 ± 11.1	89.1 ± 11.7	90.3 ± 12.5
Hip circumference (cm)	105.5 ± 7.3	105.4 ± 7.7	102.8 ± 7.6	103.7 ± 8.0	101.5 ± 7.8	102.4 ± 8.3
Waist to hip ratio	0.86 ± 0.06	0.86 ± 0.06	0.88 ± 0.06	0.88 ± 0.06	0.88 ± 0.07	0.88 ± 0.07
Percent body fat (%)	36.3 ± 5.8	36.1 ± 6.9	34.5 ± 6.2	35.4 ± 6.3*	33.5 ± 7.1	34.8 ± 8.1

Data are unadjusted mean ± SD.
BMI, body mass index; CaDiet, Dietary Calcium group; CaSuppl, Supplemental Calcium group.
* Denotes significant differences within CaDiet at 12-months from baseline for BMI (P=0.04), weight (P=0.01), and percent body fat (P=0.03).

Table 6.5 Association of total milk product, milk, yogurt or cheese with selected inflammatory and bone biomarkers at baseline and at 12 months

	Baseline				12-month			
	Unadjusted model		Adjusted model[1]		Unadjusted model		Adjusted model[2]	
Milk product (servings/wk)[3]	**ß ± SE**	***P***	**ß ± SE**	***P***	**ß ± SE**	***P***	**ß ± SE**	***P***
log hsCRP								
Total	0.009 ± 0.013	0.48	0.006 ± 0.011	0.55	-0.008 ± 0.013	0.55	-0.016 ± 0.014	0.28
Milk	0.024 ± 0.023	0.30	0.013 ± 0.019	0.52	-0.006 ± 0.027	0.81	-0.022 ± 0.022	0.32
Yogurt	-0.021 ± 0.047	0.65	0.001 ± 0.023	0.96	-0.061 ± 0.034	0.08	-0.051 ± 0.028	0.07
Cheese	0.005 ± 0.026	0.84	0.010 ± 0.022	0.66	0.002 ± 0.037	0.95	0.031 ± 0.030	0.30
log IL-6								
Total	-0.002 ± 0.004	0.63	-0.003 ± 0.004	0.46	0.003 ± 0.007	0.12	0.0003 ± 0.005	0.96
Milk	0.007 ± 0.009	0.42	0.005 ± 0.008	0.57	0.013 ± 0.012	0.29	-0.001 ± 0.008	0.95
Yogurt	-0.006 ± 0.010	0.58	-0.006 ± 0.010	0.53	0.020 ± 0.015	0.20	-0.003 ± 0.011	0.77
Cheese	-0.026 ± 0.028	0.34	-0.009 ± 0.010	0.36	0.015 ± 0.016	0.33	0.006 ± 0.011	0.60
log HMW adiponectin								
Total	0.009 ± 0.013	0.48	0.006 ± 0.011	0.55	-0.008 ± 0.013	0.55	-0.016 ± 0.014	0.28
Milk	0.021 ± 0.012	0.09	0.020 ± 0.012	0.08	-0.002 ± 0.012	0.84	0.003 ± 0.013	0.84
Yogurt	0.022 ± 0.016	0.17	0.008 ± 0.015	0.60	0.015 ± 0.015	0.31	0.023 ± 0.017	0.16
Cheese	0.025 ± 0.014	0.08	0.012 ± 0.014	0.39	0.005 ± 0.015	0.75	0.014 ± 0.018	0.43
log Total adiponectin								
Total	**0.017 ± 0.007**	**0.01**	0.012 ± 0.007	0.09	0.003 ± 0.007	0.64	0.011 ± 0.009	0.22
Milk	0.018 ± 0.012	0.13	0.015 ± 0.011	0.18	-0.004 ± 0.012	0.75	0.006 ± 0.013	0.65
Yogurt	0.024 ± 0.013	0.07	0.009 ± 0.013	0.49	0.015 ± 0.015	0.30	0.031 ± 0.016	0.06
Cheese	0.024 ± 0.013	0.07	0.011 ± 0.013	0.41	-0.004 ± 0.015	0.77	0.008 ± 0.018	0.64
log Leptin								
Total	-0.001 ± 0.010	0.94	0.002 ± 0.007	0.82	-0.0002 ± 0.010	0.99	0.007 ± 0.010	0.49
Milk	0.029 ± 0.017	0.09	0.018 ± 0.013	0.15	0.035 ± 0.018	0.06	0.027 ± 0.015	0.07
Yogurt	-0.022 ± 0.020	0.29	-0.016 ± 0.015	0.31	-0.040 ± 0.023	0.09	-0.032 ± 0.019	0.10
Cheese	-0.020 ± 0.020	0.32	-0.003 ± 0.015	0.84	-0.017 ± 0.024	0.49	0.015 ± 0.020	0.46

25(OH)D								
Total	0.73 ± 0.25	**0.004**	0.67 ± 0.25	**0.009**	0.45 ± 0.22	**0.04**	0.34 ± 0.21	0.11
Milk	0.44 ± 0.41	0.35	0.42 ± 0.47	0.37	0.64 ± 0.41	0.12	0.64 ± 0.39	0.10
Yogurt	1.36 ± 0.53	**0.01**	1.32 ± 0.53	**0.01**	0.42 ± 0.53	0.43	0.35 ± 0.51	0.49
Cheese	1.23 ± 0.52	**0.02**	1.03 ± 0.52	0.05	0.005 ± 0.57	0.99	-0.32 ± 0.54	0.55
log PTH								
Total	-0.004 ± 0.004	0.34	-0.003 ± 0.004	0.55	-0.006 ± 0.004	0.10	-0.004 ± 0.004	0.24
Milk	-0.012 ± 0.007	0.10	-0.013 ± 0.007	0.09	-0.011 ± 0.007	0.12	-0.008 ± 0.007	0.23
Yogurt	0.002 ± 0.009	0.85	0.006 ± 0.009	0.54	0.007 ± 0.009	0.42	0.01 ± 0.009	0.24
Cheese	-0.003 ± 0.008	0.76	0.001 ± 0.009	0.90	-0.004 ± 0.010	0.67	-0.001 ± 0.010	0.89
log BSAP								
Total	-0.010 ± 0.006	0.07	-0.008 ± 0.005	0.16	-0.002 ± 0.005	0.67	-0.0001 ± 0.006	0.99
Milk	-0.009 ± 0.010	0.39	-0.008 ± 0.010	0.40	-0.007 ± 0.008	0.43	-0.0001 ± 0.009	0.99
Yogurt	-0.023 ± 0.012	0.06	-0.017 ± 0.011	0.13	-0.003 ± 0.011	0.78	-0.003 ± 0.011	0.79
Cheese	-0.010 ± 0.011	0.37	-0.004 ± 0.011	0.69	0.003 ± 0.011	0.76	0.003 ± 0.012	0.80
CTX								
Total	-0.001 ± 0.002	0.75	-0.001 ± 0.002	0.74	0.002 ± 0.002	0.29	0.003 ± 0.003	0.26
Milk	-0.001 ± 0.004	0.82	0.001 ± 0.004	0.79	-0.0004 ± 0.004	0.91	0.002 ± 0.004	0.67
Yogurt	-0.002 ± 0.005	0.65	-0.003 ± 0.005	0.54	0.007 ± 0.004	0.12	0.007 ± 0.005	0.19
Cheese	0.0002 ± 0.005	0.97	-0.002 ± 0.006	0.70	0.005 ± 0.005	0.31	0.002 ± 0.005	0.69
Sclerostin								
Total	**0.2 ± 0.1**	**0.03**	0.2 ± 0.1	0.06	0.1 ± 0.1	0.21	0.2 ± 0.1	0.18
Milk	0.2 ± 0.2	0.35	0.1 ± 0.2	0.56	0.2 ± 0.2	0.31	0.2 ± 0.2	0.35
Yogurt	**0.6 ± 0.2**	**0.02**	**0.6 ± 0.2**	**0.01**	0.2 ± 0.3	0.53	0.2 ± 0.3	0.43
Cheese	0.2 ± 0.2	0.30	0.2 ± 0.2	0.49	0.3 ± 0.3	0.35	0.2 ± 0.3	0.48

25(OH)D, 25-hydroxy-vitamin D; BSAP, bone specific alkaline phosphatase; CTX, cross-linked carboxyl-terminal peptide; HMW adiponectin, high molecular weight adiponectin; IL-6, interleukin-6; PTH, parathyroid hormone.

[1] Separate models for each milk variable at baseline for IL-6 were adjusted for age and BMI. Models for HMW adiponectin, total adiponectin, and leptin were adjusted for age, BMI, 25(OH)D, and physical activity. Models for BSAP, CTX and sclerostin were adjusted for age, BMI, PTH, 25(OH)D, and physical activity. Models for PTH were adjusted for age, BMI,and physical activity. Models for 25(OH)D were adjusted for age, BMI, PTH, and physical activity.

[2] Models at 12 months were further adjusted for the assigned treatment group from the models used at baseline.

[3] Total milk product intake equaled the sum of the numbers of servings of milk, yogurt and cheese. Serving size for each item: milk (250 ml), yogurt (175 g), cottage cheese (250 ml), other cheese (50 g).

Figure 6.1 Consolidated Standards of Reporting Trials (CONSORT) diagram depicting the flow of participants. CaSuppl, Supplemental Calcium group; CaDiet, Dietary Calcium group. [a] On October 27 2015, the randomization ratio was changed from 1:1:1 to 4:4:1 due to low recruitment rate. [b] On November 22 2016, the randomization into the Control group was discontinued due to low recruitment rate and the randomization ratio was changed from 4:4:1 to 3:3.

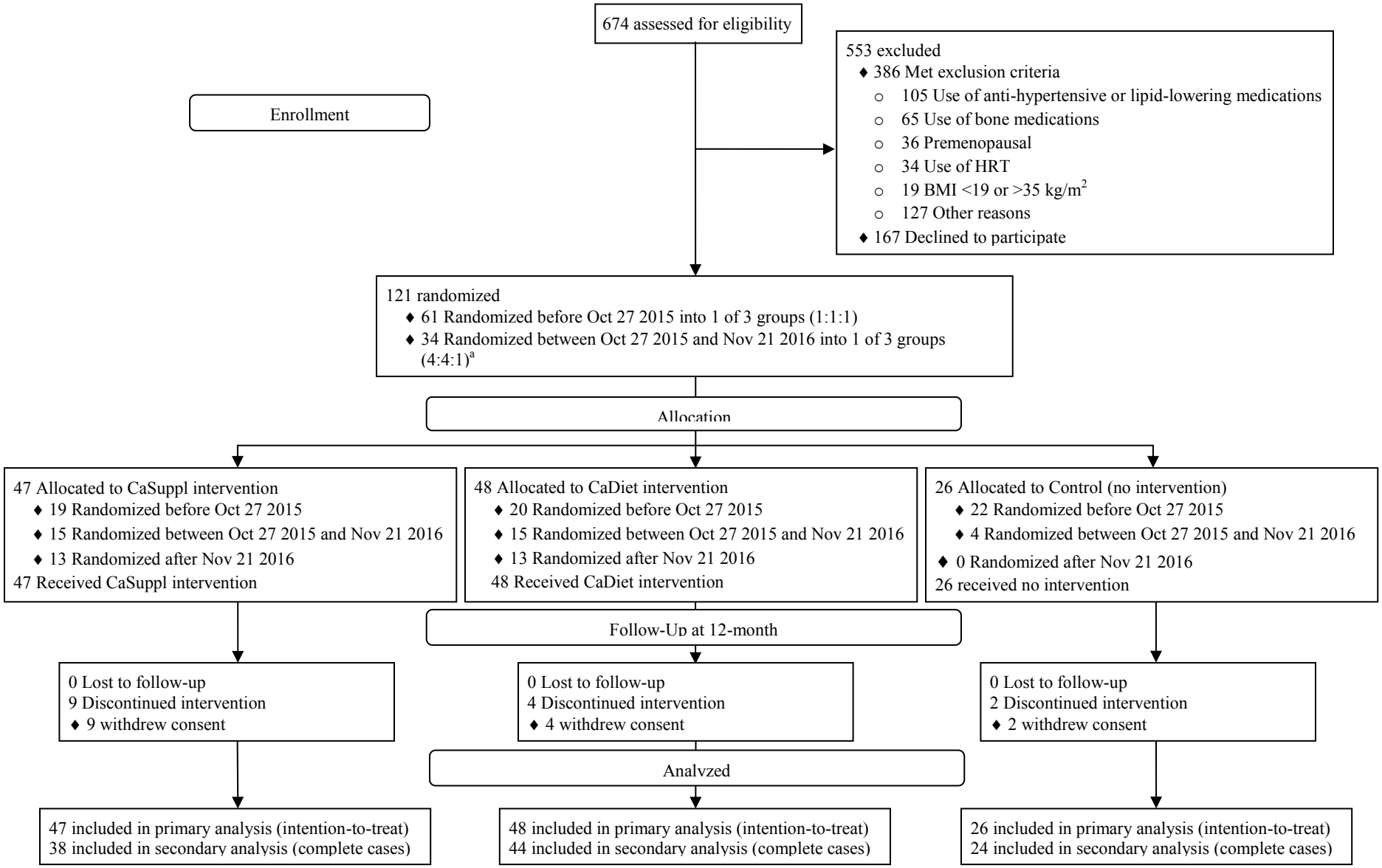
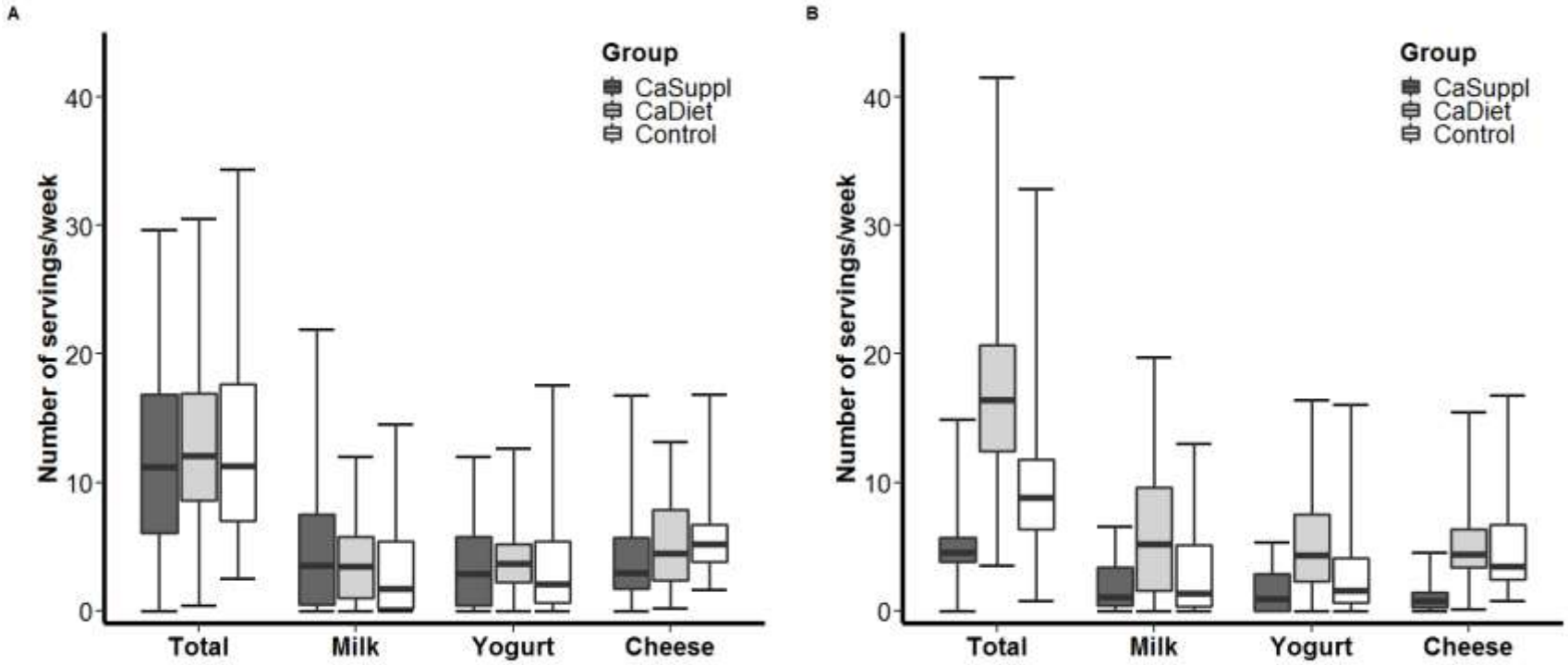


Figure 6.2 Consumption of total milk products, milk, yogurt and cheese by group at baseline and at 12 months.



Data are medians (solid line) and interquartile ranges (range of box) of the number of servings per week of milk, yogurt and cheese at baseline (A) and at 12-months (B). The whiskers show the maximum and minimum values. Intakes were estimated using a 30-day calcium-focused semi-quantitative food frequency questionnaire. Data of 121 participants are presented for baseline (CaSuppl *n*=47, CaDiet *n*=48, Control *n*=26) and those of 106 participants are presented for 12-months (CaSuppl *n*=38, CaDiet *n*=44, Control *n*=24).

CHAPTER 7

Discussion

7.1 Main outcomes and hypotheses

The overall goal of this thesis was to: (1) assess the feasibility of controlled dietary and supplemental interventions to test the effect of supplemental calcium as compared to dietary calcium on vascular and bone health outcomes in healthy postmenopausal women; (2) develop and evaluate the validity of a calcium-focused food frequency questionnaire (FFQ) to estimate usual dietary calcium and vitamin D intake of Canadian postmenopausal women; (3) investigate the association of fermented milk product (FMP) with bone health indicators in postmenopausal women or women ≥50 years; and (4) examine the effect of calcium, either from the diet alone or predominantly from the supplements, at the Recommended Dietary Allowance (RDA) level, on selected inflammatory and bone health biomarkers in healthy postmenopausal women. The 4 original papers presented in this thesis (Chapters 3-6) allowed for appropriate investigation of the objectives listed above and contributed to advances in research regarding the relationship between calcium supplementation and vascular risk. This chapter highlights the major findings pertaining to each research question of this thesis and includes a discussion on the limitations of this research work, as well as important considerations as related to future research and public health policies.

Hypothesis 1: Feasibility study

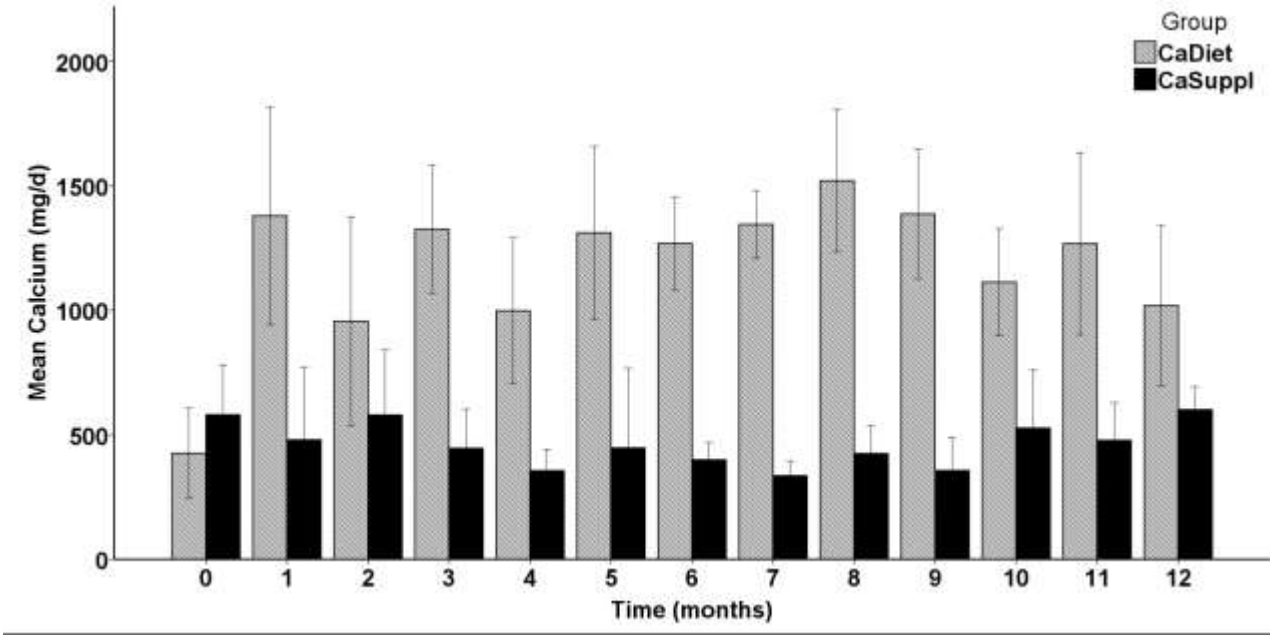
It was hypothesized that high compliance to diet modifications and supplemental interventions would be achieved through monthly nutritional counseling, and the dietary intervention would associate with better outcomes such as reduced arterial stiffness, blood pressure, and blood lipid profile.

Study 1 (Chapter 3) examined the feasibility of controlled dietary and supplemental interventions for 12 months through monthly nutritional counseling with a dietitian. The

interventions required the participants to either increase their consumption of calcium-rich foods to 3 servings/day, or to restrict it to 1 small serving/day with daily calcium supplementation. Over the trial period, both intervention arms were compliant with the assigned study interventions as shown in **Figure 7.1** (dietary adherence rate: CaSuppl 94-113% and CaDiet 95-116%; adherence rate to calcium supplement 85%, adherence rate to vitamin D supplement 98%). None of the participants reported adverse events from the dietary modifications. Preliminary results from this pilot trial did not show differences in selected surrogate markers of cardiovascular disease (CVD), body composition or serum biomarkers between groups.

During the study design phase, the potential challenges that may arise from the dietary and supplemental interventions were considered. These interventions may be demanding for some individuals, especially those who have a strong preference for milk and milk products and randomized into the supplemental group, as well as those with a usual low calcium intake who would need to increase their consumption of calcium-rich food if randomized to the food-based intervention group. To ensure adequate adherence to the allocated intervention, all participants received one-on-one counseling from a dietitian at the baseline visit and then on a monthly basis during their telephone follow-ups. The overall high adherence to both study interventions demonstrated the feasibility of the study design.

Figure 7.1 Monthly mean dietary calcium intakes by group. Data are means with standard deviations.



Results from the post-study survey indicated that participants felt adequately informed on how to modify their intake from the baseline nutritional education session and monthly follow-ups (**Appendix 1 and 2**). However, as predicted during the design phase of the study, restriction to 1 small serving/day of calcium rich food represented as a challenge from the start of the trial for some participants. Some women also experienced difficulties with measuring and estimating the quantity of food consumed. Information sheets and educational tools were subsequently created for the main randomized controlled trial (RCT) to reduce the burden on the participants and to ensure low attrition rate (**Appendix 3**).

The administration of the Harvard-Willett FFQ (552) may have also been conceptually challenging for participants due to its length and inclusion of foods from all food groups. Although the Harvard-Willett FFQ includes a section on milk and dairy products, it did not include some commonly consumed calcium-rich foods in the market place such as Greek yogurts or mixed dishes prepared with milk products. Moreover, it did not discriminate the types of cheese which have varying calcium content. Thus, a short comprehensive calcium-focused FFQ may improve the estimation of usual calcium intake and likely decrease respondent burden.

This pilot trial was also designed to assess the overall feasibility of the study design and to explore the effects of supplemental calcium and dietary calcium on selected markers of vascular and bone health. The exploratory findings suggest that in healthy normotensive postmenopausal women there are no differential effects between the two sources of calcium on vascular or bone health markers. The rigorous design of this trial differentiates it from other previous RCTs that investigated the effect of calcium on vascular or bone health outcomes. Other than the regular contacts between the dietitian and the participants, the interventions were specifically designed to provide a total calcium intake that conforms to the RDA for healthy

women >50 years. Conversely, previous trials reported administration of 1000-1200 mg/d of elemental calcium on average to treatment arms (11, 98, 316, 344, 345). Moreover, a surrogate marker of CVD, carotid-femoral pulse wave velocity, was explored as the primary outcome of the study. This represented a novelty because no previous research work, including the RCTs found in the previously published meta-analyses, has examined cardiovascular outcomes as the primary variables (8, 14, 99).

Despite its small sample size and exploratory nature of its design, this study contributed preliminary evidence on the differential effects of supplemental calcium versus dietary calcium on markers of vascular and bone health in postmenopausal women. To our knowledge, this was the first RCT that aimed at comparing the effect of the two sources of calcium on markers of vascular health as primary variables.

Study 1 ascertained that nutritional counseling at baseline and subsequently on a monthly basis to be effective at ensuring high compliance to study protocol, demonstrating the feasibility of the interventions in a larger RCT. Although limited by its small sample size, the findings of this feasibility study suggest that dietary calcium alone or in combination with supplemental calcium, at the RDA level, do not exert differential effects on the selected vascular or bone health markers in healthy postmenopausal women. Evidence from an adequately powered RCT is required to confirm these findings and to facilitate the development of public health recommendations regarding calcium supplementation.

Hypothesis 2: Calcium-focused food frequency questionnaire validation study

It was hypothesized that the semi-quantitative calcium-focused food frequency questionnaire would be in agreement with the reference method in the assessment of dietary calcium intake.

Study 2 aimed to develop and evaluate the validity of a calcium-focused FFQ to estimate usual dietary calcium intake of postmenopausal women in Montreal. This FFQ was designed for the purpose of estimating dietary calcium intake of postmenopausal women in a RCT (Study 4). We hypothesized that a semi-quantitative calcium-focused FFQ adapted from the Canadian Multicentre Osteoporosis Study (CaMOS) FFQ could be used to estimate usual dietary calcium intake in women ≥50 years old from Montreal, Canada. This study's objectives were to test the hypothesis by evaluating the relative validity of the FFQ in comparison with four 24-hour dietary recalls, and its reproducibility in comparison with a second FFQ after a 1-month interval.

The original CaMOS FFQ was expanded to include calcium-rich foods that were reported to be regularly consumed by postmenopausal women based on results from Study 1 as well as major dietary sources of vitamin D. Women in this study had a median usual dietary calcium intake of 854 mg/d (IQR 666-1068), which is consistent with the population based data (3). Contribution of dietary calcium from dairy sources was 75%, which is very similar to the patterns derived from national health surveys (67). In contrast to the Harvard-Willett FFQ, the FFQ in Study 2 included separate questions for different types of each individual milk product. This allowed a better estimation of calcium intake considering that, for example, hard cheeses provide more calcium than soft cheeses per serving.

Although the FFQ demonstrated good reproducibility (r_s=0.72, P<0.001), the estimated calcium intake from FFQ1 was significantly higher than FFQ2. There may be different sources of response bias as FFQ1 was administered in person whereas FFQ2 was completed by telephone. Nonetheless, the 51-item calcium-focused FFQ had a moderate agreement with the reference method (κw=0.55). This was in line with another validation study of a calcium-focused FFQ that was conducted in Croatian postmenopausal women (553). The relative agreement between the

FFQ and the 24HRs was within the range reported in similar studies in women (379, 404, 407). The FFQ underestimated dietary calcium intake by a relatively small amount (<100 mg/d) that would be equivalent to approximately one-third of a serving of *Milk and Alternatives* according to the 2007-2019 Canada's Food Guide. This level of underestimation could misclassify some individuals into adjacent quantiles, but only 2.8% of the participants in this study were misclassified into extreme quartiles.

The 51-item semi-quantitative FFQ is a useful tool to estimate dietary calcium intakes in postmenopausal women. The quantitative information on the relative validity of the 51-item FFQ substantially enhances the interpretation of the data pertaining to calcium intake from the Calcium Study (Study 4) to investigate the effect of dietary calcium interventions on health outcomes.

Hypothesis 3: Systematic review and meta-analysis of fermented milk products on bone health

It was hypothesized that fermented milk product consumption would associate with more benefits to bone health, such as greater bone mineral density, lower incidence of fragility fractures, and lower levels of bone turnover markers.

In Study 3, a systematic review and meta-analysis was conducted to examine the association between FMPs with bone health outcomes in postmenopausal women. Three RCTs, 3 prospective cohorts, and 3 case-control studies were included in the review. Data from prospective cohort studies suggest that higher yogurt consumption was associated with reduced hip fracture risk. However, no association between cheese intake and hip fracture risk was found. This may be due to the relatively small number of studies and incident fracture events to detect an association. With the exception of the null effect of cheese, the results of this meta-analysis

are consistent with the findings of another meta-analysis that examined the relationship between milk product consumption and hip fracture risk (415). Case-control studies revealed that cheese intake had either a null or a protective effect against osteoporosis, but no association was found with yogurt consumption. RCTs of FMPs were generally short-term (<2 months), but all 3 included studies reported a reduction in bone resorption markers. In line with previous observations, the inverse association between FMP consumption and bone resorption markers has previously been reported in large cross-sectional studies (470, 471). The classification of all cheese as 1 category in these studies may have also influenced the results, considering the large variety of types of cheese that differ in their fermentation process as well as their nutrient profiles.

The overall quality of evidence was very low due to the limited data available and most studies were of observational design. Although the results related to the protective effect of yogurt consumption on hip fracture risks are consistent with those of another report (415), it was evident that the Swedish Mammography Cohort had a major influence on the significance of the effect estimate (452). Taken into consideration the dietary and genetic differences between cohorts from different countries, the beneficial effect of yogurt requires further investigation in interventional studies. As the proposed mechanism underlying the beneficial effect of FMP may be the modulation of inflammation, investigation in changes of a panel of inflammatory markers would help fill the knowledge gap related to the health effects of FMPs.

Although the results of this study were unable to compare the benefits of FMP versus non-FMP on bone health indicators, the findings are consistent with the hypothesis of the varying health benefits of each dairy product as a result of their distinct nutrient matrices. Only greater yogurt consumption was associated with a reduced risk of hip fracture compared with

low or no intake. Daily cheese intake may be associated with higher BMD T-scores, but evidence was limited. These results add to the literature on the potential benefits of yogurt consumption on bone health in postmenopausal women, although the quality of evidence is low. Additional and longer-term trials examining these relations are warranted to guide dietary recommendations regarding the benefits of FMP on bone health.

Hypothesis 4: Effect of calcium on selected inflammatory and bone biomarkers

It was hypothesized that the dietary calcium intervention would be associated with a more favourable effect on markers of inflammation and bone health markers than a calcium intake predominantly derived from supplements.

In the final manuscript (Chapter 6), the effect of calcium at the level of the RDA, either from dietary sources alone or predominantly from supplements, on selected markers of inflammation and bone metabolism was investigated in healthy postmenopausal women. As part of a 12-month RCT, blood samples were collected at baseline and at the 12-month final visit.

At 12 months, neither of the 2 interventions caused a change in the selected inflammatory markers hsCRP, IL-6, adiponectin or leptin, and there were no between-group differences. There were also no association between individual milk product intake and the selected inflammatory markers. These findings are consistent with those from other RCTs of short-term supplemental calcium and dairy interventions (43, 291-294, 529, 530). This body of evidence provides strong evidence to refute the hypothesis of supplemental calcium inducing inflammation as part of the underlying mechanism causing CVD. Although milk, yogurt, or cheese intakes were not associated with markers of inflammation, the intakes (<3 servings/day) were too low to draw strong conclusions.

Serum 25-hydroxyvitamin D [25(OH)D] significantly increased in all study arms as

expected due to the high adherence with study supplements. The observed decrease in serum CTX in both treatment arms and lack of change in the control arm indicates that supplemental and dietary calcium at the RDA level have similar effects on bone turnover and exert beneficial skeletal actions by decreasing bone resorption. Although these findings may be limited to vitamin D sufficient healthy postmenopausal women, these results are consistent with other interventional studies of dietary or supplemental calcium intervention in postmenopausal women (486, 523-525). BMD measurements were not included as outcomes in this trial because a significant change after one year of calcium and vitamin D supplementation would be unlikely. Most osteoporosis therapies do not cause large increases in BMD and repeated BMD measurements sooner than 18-24 months following intervention are not recommended (554). However, the addition of a baseline BMD measurement may identify women with undiagnosed osteoporosis, considering that most cases of osteoporosis are often diagnosed following a fracture (555). Data on baseline BMD could allow further exploration into the interaction between the nutrition interventions, BMD, inflammatory biomarkers and markers of bone metabolism.

An increase in serum ionized calcium, PTH, or sclerostin concentrations would support the speculated mechanism of vascular calcification induced by calcium supplementation. However, there were no between-group differences in these markers at 12 months. Few studies have investigated the potential mechanism of vascular calcification and their findings also did not support this hypothesis. A recent animal study designed to examine whether a high consumption of calcium similar to human calcium intake at the Tolerable Upper Intake Level (UL), as supplemental calcium (in the form of calcium carbonate) or as non-fat dairy, led to an acceleration of vascular calcification in Ossabaw miniature swine after 6 months (532). This

animal model possesses similarities to humans in lipid metabolism (556), and also has a genetic predisposition to develop metabolic syndrome and coronary artery disease when fed an atherogenic diet for several months (557, 558). The investigators were unable to detect an effect of high calcium diets from either supplemental or dietary sources on coronary artery calcium deposition in their animal model. Thus, their findings do not support the hypothesis that calcium supplementation increases the cardiovascular risk through the acceleration of calcium deposition in the coronary arteries. An ancillary study was conducted to examine the effect of 1.2 g of elemental calcium as calcium carbonate daily or a matched placebo on carotid intima-media thickness (cIMT) and carotid atherosclerosis in elderly women >70 years (13). Following 3 years of supplementation, no adverse effect of calcium supplementation on cIMT or atherosclerosis compared to placebo was observed, both before and after adjusting for baseline cardiovascular risk factors. Preliminary findings of the Calcium Study, the main RCT as described in Chapter 6 and led by our research group, also did not detect differential effects on surrogate markers of vascular health and atherosclerosis, measured as carotid-femoral pulse wave velocity (cfPWV) and cIMT, respectively (498). Taken together with the findings presented in this thesis, the current body of evidence indicates that calcium intake at the RDA level and below the UL, regardless of the source, does not increase the risk of vascular calcification over 1 year.

In terms of changes in body composition, the CaDiet arm displayed an increase in body weight, BMI, and percent body fat at 12 months. Although the observed weight gain was not considered as clinically significant, this result contradicts with the evidence derived from dairy intervention studies. Most of these studies have shown body weight reduction and increase in lean body mass following dairy interventions compared to control (559, 560). However, those interventions were usually combined with hypocaloric regimens to promote weight loss. In our

study, participants were instructed to increase their intake of dietary calcium by including 3 servings/day of calcium-rich foods, and milk and milk products were the main contributors. It is possible that the observed weight gain and increase in BMI and percent body fat in the CaDiet arm resulted from an increase in energy intake from adding calcium-rich foods into the diet instead of substituting for other foods. Energy intake was higher in the CaDiet arm compared to the CaSuppl arm throughout the study, but the difference (~300 kcal/day) was not excessively more. Due to the limited data on dietary intakes before the trial, we were unable to compare the usual dietary intake prior to the trial to the overall intake during the study to assess whether energy intake has increased in the CaDiet arm.

Overall, this study adds to the body of evidence supporting the hypothesis that calcium intakes that meet current dietary recommendations, either from the diet alone or combined with supplements, do not influence markers of inflammation healthy postmenopausal women. Whether exceeding the recommended intakes poses a cardiovascular risk, especially in individuals with predisposed vascular risks and chronic systemic inflammation, requires further investigation. Nonetheless, as per the current guidelines, calcium requirements should first be met through the diet in order to benefit from a variety of bone-building nutrients, and supplements should be considered when intake from the diet is insufficient.

7.2 Strengths and limitations

This thesis provided a comprehensive understanding on the differential effects of supplemental and dietary calcium on selected markers involved in inflammation and bone metabolism in healthy postmenopausal women. Study 4 was part of an RCT that was designed to estimate the effect of supplemental calcium versus dietary calcium on surrogate and subclinical markers of CVD. This RCT was the first trial investigating the effect of the two different sources

of calcium compared to a control group on vascular endpoints as primary outcomes. In addition to the controlled nutritional interventions designed to meet the RDA for calcium, the use of objective markers was also a strength of the study.

To quantify usual dietary calcium intake, Study 4 used a 30-day 51-item calcium-focused FFQ that was designed and validated in postmenopausal women. This interviewer-administered FFQ required less time to complete than the Harvard-Willett FFQ which was used in the pilot trial. Although the calcium-focused FFQ was unable to estimate usual energy intakes, it captured the intake of calcium from trending calcium-rich items that were not included in the Harvard-Willett FFQ. In Study 2, several statistical methods were used to evaluate the relative reproducibility, validity, sensitivity and specificity of the FFQ, which are important characteristics of a valid FFQ but not commonly performed in other validation studies. Considering the relatively low gross misclassification into extreme quartiles and that systematic bias was relatively low, the results suggest that the calcium-focused FFQ is a valid tool to discriminate low and high dietary calcium intakes in postmenopausal women and to rank dietary calcium intake in epidemiological studies. These results support its use in the RCT, in which participants were following a low or high dietary calcium intervention.

In Study 3, different tools were used to evaluate the risk of bias and methodological quality of interventional and observational studies (446, 447). Furthermore, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to assess the overall quality of evidence across studies for each outcome (448). Although the meta-analysis was unable to directly compare the effect of FMP with non-FMP on bone health, the findings were consistent with the literature suggesting benefits of FMP in human health, including bone and vascular health, though the mechanisms warrant more clinical research. The

emerging evidence highlights the need to consider each type of dairy product separately in order to provide high-level evidence to guide changes in dietary recommendations.

Study 4 was an RCT, which is considered as the gold standard for evaluating interventions. It was designed, conducted and reported according to the CONSORT statement guidelines for multi-arm parallel-group RCTs (561, 562). Allocation sequence was unpredictable and the random assignment to study arms reduced selection bias at trial entry. The inclusion of 3 trial arms allowed the comparison of calcium intake predominantly from supplements to calcium from dietary sources alone or no intervention. This was the first study with such a design and allowed a better understanding of the differential effects of the different sources of calcium. The assessment of multiple serum and plasma biomarkers to capture the potential effect of the interventions on inflammation and bone health was also a strength of Study 4. Although serum and plasma inflammatory markers reflect many tissues other than vascular and bone tissues, several trials have investigated the effect of anti-inflammatory agents on non-cardiovascular inflammatory diseases and on incidence of cardiovascular events, suggesting a causal role of inflammation on CVD. For instance, the IL-6 receptor blocker tocilizumab increased HDL particle concentrations in patients with rheumatoid arthritis compared to placebo (147). Recurrent cardiovascular events were significantly lowered in patients who had residual inflammation after myocardial infarction following the treatment with canakinumab, a human monoclonal anti-IL-1ß antibody (563). Colchicine, an anti-inflammatory treatment for gout, was found to be effective for secondary prevention of CVD (564). More trials are currently ongoing (148, 565), suggestive of the important role played by individual inflammatory markers on the pathogenesis and progression of atherosclerosis.

There is currently limited research examining the effect of calcium or dairy interventions

on serum sclerostin. Sclerostin is recognized as an inhibitor of bone formation but the significance of circulating sclerostin related to bone health and vascular calcification remains poorly understood. Different immunoassays have been used to measure sclerostin concentrations in the literature, resulting in significant inter-assay differences according to the immunoassay used due to varying capacity of the assays to detect the intact molecule and additional fragments of sclerostin (270). To date, there is no consensus on which assay to use. As such, data from Study 4 may guide future studies that choose the same method to measurement serum sclerostin concentrations.

Monthly follow-ups allowed participants to receive direct feedback from the dietitian and helped reassure the study participants throughout the study period. In general, participants are prone to adhere poorly to their assigned intervention due to study fatigue or because the intervention is genuinely difficult (566). Building on the observations from Study 1, regular contacts with the dietitian in Study 4 helped improve the participants' understanding on how to increase or decrease their dietary calcium intake and on how to estimate portion sizes. The nutritional counseling may partially explain the higher compliance rate of 95% than those reported in other calcium intervention studies (11, 98, 316, 344, 345).

This research work was not without limitations. The FFQ was validated in predominantly highly educated postmenopausal women of white descent. Despite the study being conducted in a multi-ethnic urban city in Canada, the generalizability of the findings from Study 2 requires further evaluation. More importantly, the findings from Study 4 cannot be generalized to other groups, such as premenopausal women, men, the frail elderly, patients with liver disease, or patients with CVD or osteoporosis. CVD and osteoporosis often develops or exists in the context of multi-comorbidity and frailty that would thereby influence the inflammatory process (567). A

major limitation of the RCT was its small sample size, which was estimated based on the primary vascular outcome (i.e., cfPWV) and not biomarkers of inflammation. Thus, the trial was unlikely adequately powered to determine a real effect or robustly claim the absence of an effect (568). Using data from previous reports, 671 participants would be required in each arm to detect a mean difference of 0.15 mg/L in hsCRP, or 466 participants per group to detect a mean difference of 2.35 ng/L in sclerostin concentrations to obtain a power of 80% with α set at 0.05 (43, 275).

Diverse recruitment methods were employed to maximize participant enrolment and the recruitment period was extended and took place from January 2014 to November 2017. Participants were recruited through posters, local newspaper advertisements, presentations in the community, and media interviews. Despite the recruitment efforts, recruitment rate remained low. Nonetheless, 106 participants completed the study with an attrition rate of 12.4%. Although larger trials may not be feasible, alternative populations from other countries as well as higher risk groups should be considered to confirm our findings.

Nutrition research involving the assessment of self-reported dietary intakes is prone to inaccuracies due to under- or over-reporting. For instance, in Study 2, the estimated calcium intake from FFQ1 was significantly higher than the intake estimated from FFQ2. Response bias by responding in a manner consistent with expected norms could not be ruled out (310, 399). To maximize reporting accuracy, participants were provided with resources to help them estimate the amounts of food consumed in Study 2 and Study 4. Furthermore, at the end of each telephone follow-up in Study 4, immediate feedback was provided to the participant using motivational interviewing techniques to enhance their motivation and commitment to adherence to the study intervention (569).

Although milk and milk products were the predominant sources of dietary calcium consumed in Study 4, some participants abstained from dairy consumption and relied on fortified plant-based beverages and tofu to meet their assigned calcium targets. The analyses could not be stratified by sources due to low intakes of calcium from non-dairy sources. Similarly, given that participants were restricted to their source of dietary calcium, the consumption of individual dairy product was relatively low to allow a thorough assessment of their individual effect on the selected biomarkers. Finally, the inability to blind the participants and the dietitian from the allocations was another potential limitation.

7.3 Future directions

This thesis has provided evidence to support the safety of calcium supplement use to meet the RDA in healthy postmenopausal women, yet some questions remain unanswered. Contrary to most calcium intervention studies, the dosage of 750 mg/day required the participants to limit their dietary calcium intake to 450 mg/day. This was observed to be a challenge as this involved a restriction of some preferred foods. Nonetheless, due to the lack of RCTs, studies with a similar design to the one presented in this thesis should be conducted in women with predisposed vascular risks or older frail adults with sarcopenia to examine the effect of calcium on inflammatory and surrogate markers of vascular health.

There is a growing interest in identifying the health benefits of FMP yet there are currently limited published interventional trials. Study 3 made it evident that there is a need to further investigate the effect of individual types of dairy on bone health outcomes. Although measurement of areal BMD would unlikely show significant differences after 1 year of nutrition intervention, changes may occur in the bone microstructure as determined by peripheral computed tomography (pQCT). A previous study found that age-related bone loss varied

between the trabecular and cortical compartments in postmenopausal women, where a greater rate of cortical bone loss was detected between measurements of 1-year interval (570). A prospective cohort study of 482 postmenopausal women examined the association between FMP and non-FMP (i.e., milk and ripened cheese) with bone microstructure by high resolution pQCT (HR-pQCT) measured at baseline and at 3 years (471). Their findings suggested that FMP consumption is associated with attenuated loss of radius total volumetric BMD and of cortical bone at non-bearing bone sites, whereas milk consumption associated with attenuated loss of areal BMD and of failure to load at the radius (471). Taken together, further investigation into the effect of FMP consumption on bone microstructure in future trials is needed to generate high level evidence to support the hypothesis regarding the superior health benefits of FMP.

Similarly, more work is also required to elucidate the effect of FMP and non-FMP on inflammatory markers and vascular health outcomes. Accumulating evidence has revealed that the gut microbiota plays an important role in a large variety of disorders, including atherosclerosis and osteoporosis (571). Ageing and changes to gut microbiota composition increases mucosal barrier permeability, allowing bacteria and their products into the circulatory system thereby contributing to a chronic pro-inflammatory state (572). So far, the increased gut permeability and leakage of pro-inflammatory products has only been demonstrated in animal models, but dysbiosis has been associated with conditions that become more prevalent with ageing, such as obesity and type 2 diabetes (573). Modification of the gut microbiota with prebiotics and probiotics may influence the development of metabolic diseases and reduce systemic inflammation (474, 574). Given that FMPs are accessible dietary sources of probiotics, short- and medium-term interventions comparing the effect of milk, cheese, and yogurt separately on inflammatory and bone biomarkers can help close the knowledge gap and provide

high-level evidence to guide future dietary recommendations. However, long-term trials may not be feasible as participant fatigue to the interventions (e.g., 3 servings of milk, 3 servings of cheese, or 3 servings of yogurt) will be highly likely.

7.4 Public health implication

National health survey data indicate that women >50 years do not meet the calcium requirements and use of calcium supplements have decreased following the controversy regarding their safety. Yet, osteoporosis remains a major public health burden affecting 2 million Canadians, mainly postmenopausal women and the elderly (2).

The interventions presented in this study were based on the RDA for healthy women >50 years to maintain bone integrity by slowing the rate of bone loss. Individuals should aim to meet the RDA through food sources in order to benefit from the other nutrients as part of the food matrix, and to fill the gap with a nutritional supplement. Findings from the research of this thesis add to the body of evidence from intervention studies supporting the lack of detrimental effect on vascular calcification and its positive effect on bone turnover. Thus, given the lack of high level evidence demonstrating the adverse effects on the vasculature, supplemental calcium should remain as an option for those who are unable to meet the RDA from food sources alone. However, in clinical practice, it remains important to evaluate the patient's usual dietary calcium intake prior to prescribing a calcium supplement.

An important change to the Canada's Food Guide also requires careful consideration and attention. As of January 2019, the Canada's Food Guide has changed its approach to promote healthy eating. To make healthy eating easier for Canadians, the guide no longer recommends foods as food groups nor how much to eat. Instead, Canadians are encouraged to 'have plenty of vegetable and fruits', 'eat protein foods', and 'choose whole grain foods'. As a result, milk and

milk alternatives are grouped within the protein group along with meat, pulses, legumes, nuts and seeds. An analysis of the nutrient content of foods depicted in the Canada's Food Guide's Snapshot showed a high probability of inadequacy, ranging up to >90%, in calcium and vitamin D intakes for most age and sex groups (575). Although the estimated content of calcium was based on a number of assumptions, these results suggest that further guidance is required by users of the new food guide in order to achieve the recommended intakes for calcium.

Thus, calcium from whole foods, such as milk and milk products, should not be discouraged and perhaps fermented milk products should be encouraged though further research is needed to provide empirical evidence to demonstrate their benefits and varying effects on bone health and cardiovascular health. In the same vein, calcium supplements remain a low-cost and safe option for healthy postmenopausal women to meet the recommendations for bone health.

7.5 Conclusions

In summary, this thesis has shown that calcium consumption at the RDA level, from the diet alone or predominantly from supplements, does not have an effect on inflammatory markers in healthy postmenopausal women after 12 months. However, both interventions decreased bone turnover markers at 12 months whereas no change was observed in the Control arm. This body of work significantly contributes to the growing evidence supporting the safety of both supplemental and dietary sources of calcium in healthy postmenopausal women. Our meta-analysis suggested that higher consumption of yogurt may be favorable to bone health, but this could not be confirmed in our analyses. Future nutrition trials of fermented milk products as compared to non-fermented milk consumption on bone outcomes, ascertained through new imaging modalities, such as HR-pQCT, will provide a better understanding on whether current recommendations require updates by promoting fermented milk products.

References

1. Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, Tenenhouse A, Davison KS, Josse RG, Prior JC et al. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. *J Bone Miner Res.* 2010;25(9):1948-1957.
2. Osteoporosis Canada. Fast facts. Internet: https://osteoporosis.ca/about-the-disease/fast-facts/. Accessed January 3, 2020.
3. Garriguet D. Bone health: osteoporosis, calcium and vitamin D. *Health Rep.* 2011;22(3):7-14.
4. Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, Sempos CT, Picciano MF. Estimation of total usual calcium and vitamin D intakes in the United States. *J Nutr.* 2010;140(4):817-822.
5. Chung M, Lee J, Terasawa T, Lau J, Trikalinos TA. Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force. *Ann Intern Med.* 2011;155(12):827-838.
6. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet.* 2007;370(9588):657-666.
7. Wallace RB, Wactawski-Wende J, O'Sullivan MJ, Larson JC, Cochrane B, Gass M, Masaki K. Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements. *Am J Clin Nutr.* 2011;94(1):270-277.
8. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ.* 2010;341:c3691.
9. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ.* 2011;342:d2040.
10. Shah SM, Carey IM, Harris T, DeWilde S, Cook DG. Calcium supplementation, cardiovascular disease and mortality in older women. *Pharmacoepidemiol Drug Saf.* 2010;19(1):59-64.
11. Lewis JR, Calver J, Zhu K, Flicker L, Prince RL. Calcium supplementation and the risks of atherosclerotic vascular disease in older women: results of a 5-year RCT and a 4.5-year follow-up. *J Bone Miner Res.* 2011;26(1):35-41.
12. Prentice RL, Pettinger MB, Jackson RD, Wactawski-Wende J, Lacroix AZ, Anderson GL, Chlebowski RT, Manson JE, Van Horn L, Vitolins MZ et al. Health risks and benefits from calcium and vitamin D supplementation: Women's Health Initiative clinical trial and cohort study. *Osteoporos Int.* 2013;24(2):567-580.
13. Lewis JR, Zhu K, Thompson PL, Prince RL. The effects of 3 years of calcium supplementation on common carotid artery intimal medial thickness and carotid atherosclerosis in older women: an ancillary study of the CAIFOS randomized controlled trial. *J Bone Miner Res.* 2014;29(3):534-541.
14. Lewis JR, Radavelli-Bagatini S, Rejnmark L, Chen JS, Simpson JM, Lappe JM, Mosekilde L, Prentice RL, Prince RL. The effects of calcium supplementation on verified coronary heart disease hospitalization and death in postmenopausal women: a

collaborative meta-analysis of randomized controlled trials. *J Bone Miner Res.* 2015;30(1):165-175.
15. Harvey NC, D'Angelo S, Paccou J, Curtis EM, Edwards M, Raisi-Estabragh Z, Walker-Bone K, Petersen SE, Cooper C. Calcium and vitamin D supplementation are not associated with risk of incident ischemic cardiac events or death: findings from the UK Biobank Cohort. *J Bone Miner Res.* 2018;33(5):803-811.
16. Praagman J, Franco OH, Ikram MA, Soedamah-Muthu SS, Engberink MF, van Rooij FJ, Hofman A, Geleijnse JM. Dairy products and the risk of stroke and coronary heart disease: the Rotterdam Study. *Eur J Nutr.* 2015;54(6):981-990.
17. Huth PJ & Park KM. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Adv Nutr.* 2012;3(3):266-285.
18. Khan B, Nowson CA, Daly RM, English DR, Hodge AM, Giles GG, Ebeling PR. Higher dietary calcium intakes are associated with reduced risks of fractures, cardiovascular events, and mortality: a prospective cohort study of older men and women. *J Bone Miner Res.* 2015;30(10):1758-1766.
19. Gibson RA, Makrides M, Smithers LG, Voevodin M, Sinclair AJ. The effect of dairy foods on CHD: a systematic review of prospective cohort studies. *Br J Nutr.* 2009;102(9):1267-1275.
20. Larsson SC, Virtamo J, Wolk A. Dairy consumption and risk of stroke in Swedish women and men. *Stroke.* 2012;43(7):1775-1780.
21. Tavani A, Gallus S, Negri E, La Vecchia C. Milk, dairy products, and coronary heart disease. *J Epidemiol Community Health.* 2002;56(6):471-472.
22. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. *J Am Coll Nutr.* 2010;29(4):357-364.
23. Elwood PC, Givens DI, Beswick AD, Fehily AM, Pickering JE, Gallacher J. The survival advantage of milk and dairy consumption: an overview of evidence from cohort studies of vascular diseases, diabetes and cancer. *J Am Coll Nutr.* 2008;27(6):723s-734s.
24. Larsson SC, Orsini N, Wolk A. Dietary calcium intake and risk of stroke: a dose-response meta-analysis. *Am J Clin Nutr.* 2013;97(5):951-957.
25. Da Silva MS & Rudkowska I. Dairy products on metabolic health: current research and clinical implications. *Maturitas.* 2014;77(3):221-228.
26. Rautiainen S, Wang L, Manson JE, Sesso HD. The role of calcium in the prevention of cardiovascular disease — a review of observational studies and randomized clinical trials. *Curr Atheroscler Rep.* 2013;15(11):362.
27. Dong JY, Szeto IM, Makinen K, Gao Q, Wang J, Qin LQ, Zhao Y. Effect of probiotic fermented milk on blood pressure: a meta-analysis of randomised controlled trials. *Br J Nutr.* 2013;110(7):1188-1194.
28. Rice BH, Quann EE, Miller GD. Meeting and exceeding dairy recommendations: effects of dairy consumption on nutrient intakes and risk of chronic disease. *Nutr Rev.* 2013;71(4):209-223.
29. da Silva Ferreira T, Torres MR, Sanjuliani AF. Dietary calcium intake is associated with adiposity, metabolic profile, inflammatory state and blood pressure, but not with erythrocyte intracellular calcium and endothelial function in healthy pre-menopausal women. *Br J Nutr.* 2013;110(06):1079-1088.

30. Rice BH, Cifelli CJ, Pikosky MA, Miller GD. Dairy components and risk factors for cardiometabolic syndrome: recent evidence and opportunities for future research. *Adv Nutr.* 2011;2(5):396-407.
31. Xiao Q, Murphy RA, Houston DK, Harris TB, Chow W-H, Park Y. Dietary and supplemental calcium intakes in relation to mortality from cardiovascular diseases in the NIH-AARP Diet and Health Study. *JAMA Intern Med.* 2013;173(8):639-646.
32. Astrup A. Yogurt and dairy product consumption to prevent cardiometabolic diseases: epidemiologic and experimental studies. *Am J Clin Nutr.* 2014;99(5 Suppl):1235s-1242s.
33. Bostick RM, Kushi LH, Wu Y, Meyer KA, Sellers TA, Folsom AR. Relation of calcium, vitamin D, and dairy food intake to ischemic heart disease mortality among postmenopausal women. *Am J Epidemiol.* 1999;149(2):151-161.
34. Iso H, Stampfer MJ, Manson JE, Rexrode K, Hennekens CH, Colditz GA, Speizer FE, Willett WC. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. *Stroke.* 1999;30(9):1772-1779.
35. Hu D, Huang J, Wang Y, Zhang D, Qu Y. Dairy foods and risk of stroke: a meta-analysis of prospective cohort studies. *Nutr Metab Cardiovasc Dis.* 2014;24(5):460-469.
36. Huang LY, Wahlqvist ML, Huang YC, Lee MS. Optimal dairy intake is predicated on total, cardiovascular, and stroke mortalities in a Taiwanese cohort. *J Am Coll Nutr.* 2014;33(6):426-436.
37. Rice BH. Dairy and cardiovascular disease: a review of recent observational research. *Curr Nutr Rep.* 2014;3:130-138.
38. Patterson E, Larsson SC, Wolk A, Åkesson A. Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. *J Nutr.* 2013;143(1):74-79.
39. Michaëlsson K, Melhus H, Warensjö Lemming E, Wolk A, Byberg L. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. *BMJ.* 2013;346:f228.
40. Michaëlsson K, Wolk A, Langenskiold S, Basu S, Warensjo Lemming E, Melhus H, Byberg L. Milk intake and risk of mortality and fractures in women and men: cohort studies. *BMJ.* 2014;349:g6015.
41. Guo J, Astrup A, Lovegrove JA, Gijsbers L, Givens DI, Soedamah-Muthu SS. Milk and dairy consumption and risk of cardiovascular diseases and all-cause mortality: dose–response meta-analysis of prospective cohort studies. *Eur J Epidemiol.* 2017;32(4):269-287.
42. Labonte ME, Couture P, Richard C, Desroches S, Lamarche B. Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. *Am J Clin Nutr.* 2013;97(4):706-717.
43. Labonte ME, Cyr A, Abdullah MM, Lepine MC, Vohl MC, Jones P, Couture P, Lamarche B. Dairy product consumption has no impact on biomarkers of inflammation among men and women with low-grade systemic inflammation. *J Nutr.* 2014;144(11):1760-1767.
44. Esmaillzadeh A & Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. *Public Health Nutr.* 2010;13(9):1395-1402.
45. Zemel MB & Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. *J Nutr.* 2008;138(6):1047-1052.

46. Mozaffarian D. Dairy foods, obesity, and metabolic health: the role of the food matrix compared with single nutrients. *Adv Nutr.* 2019;10(5):917S-923S.
47. Buziau AM, Soedamah-Muthu SS, Geleijnse JM, Mishra GD. Total fermented dairy food intake is inversely associated with cardiovascular disease risk in women. *J Nutr.* 2019;149(10):1797-1804.
48. Chrysant SG & Chrysant GS. Controversy regarding the association of high calcium intake and increased risk for cardiovascular disease. *J Clin Hypertens.* 2014;16(8):545-550.
49. Heaney RP. The calcium economy. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totowa, NJ: Humana Press; 2006. p. 145-162.
50. Institute of Medicine. Dietary Reference Intakes for calcium and vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington, DC: National Academies Press (US); 2011.
51. Koeppen BM & Stanton BA. Potassium, calcium, and phosphate homeostasis. In: Koeppen BM, Stanton BA, editors. Berne & Levy Physiology. Philadelphia: Elsevier; 2018. p. 647-669.
52. Siddiqui JA & Partridge NC. Physiological bone remodeling: systemic regulation and growth factor involvement. *Physiology.* 2016;31(3):233-245.
53. Mundy GR & Guise TA. Hormonal control of calcium homeostasis. *Clin Chem.* 1999;45(8):1347-1352.
54. Zemel BS. Human biology at the interface of paediatrics: measuring bone mineral accretion during childhood. *Ann Hum Biol.* 2012;39(5):402-411.
55. Demontiero O, Vidal C, Duque G. Aging and bone loss: new insights for the clinician. *Ther Adv Musculoskelet Dis.* 2012;4(2):61-76.
56. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA.* 2001;285(6):785-795.
57. Compston JE, McClung MR, Leslie WD. Osteoporosis. *Lancet.* 2019;393(10169):364-376.
58. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for calcium and vitamin D. Washington (DC): National Academy Press; 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK56070/ doi: 10.17226/13050.
59. Health Canada. Eating well with Canada's Food Guide. 2011. Internet: https://www.canada.ca/en/health-canada/services/canada-food-guide/about/history-food-guide/eating-well-with-canada-food-guide-2007.html. Accessed December 2, 2019.
60. Canadian Food Inspection Agency. Foods to which vitamins, mineral, nutrients and amino acids may or must be added. 2018. Internet: http://www.inspection.gc.ca/food/labelling/food-labellingfor-industry/nutrient-content/reference-information/eng/1389908857542/1389908896254?chap=1. Accessed Jan 3, 2020.
61. Canadian Food Inspection Agency. Fortification. Internet: https://www.inspection.gc.ca/food-label-requirements/labelling/industry/fortification/eng/1468504433692/1468504697186. Accessed Jan 3, 2020.
62. Health Canada. Canadian Nutrient File. 2015. Internet: https://food-nutrition.canada.ca/cnf-fce/index-eng.jsp. Accessed October 18, 2018.

63. Health Canada. Eating Well with Canada's Food Guide: What is a food guide serving of Milk and Alternatives. 2007. Internet: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/fn-an/alt_formats/hpfb-dgpsa/pdf/food-guide-aliment/serving_milk-lait_portion-eng.pdf. Accessed September 29, 2019.
64. Weaver CM & Heaney RP. Food sources, supplements, and bioavailability. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totowa, NJ: Humana Press; 2006. p. 129-142.
65. Straub DA. Calcium supplementation in clinical practice: a review of forms, doses, and indications. *Nutr Clin Pract.* 2007;22(3):286-296.
66. Tugault-Lafleur CN & Black JL. Differences in the quantity and types of foods and beverages consumed by Canadians between 2004 and 2015. *Nutrients.* 2019;11(3):526.
67. Auclair O, Han Y, Burgos SA. Consumption of milk and alternatives and their contribution to nutrient intakes among Canadian adults: evidence from the 2015 Canadian Community Health Survey-Nutrition. *Nutrients.* 2019;11(8).
68. Statistics Canada. 2015 Canadian Community Health Survey (CCHS) — Nutrition: user guide. 2017. Internet: https://www.canada.ca/en/health-canada/services/food-nutrition/food-nutrition-surveillance/health-nutrition-surveys/canadian-community-health-survey-cchs/2015-canadian-community-health-survey-nutrition-food-nutrition-surveillance.html. Accessed December 2, 2019.
69. World Health Organization. Cardiovascular diseases. 2015. Internet: http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed July 21, 2015.
70. Stramba-Badiale M, Fox KM, Priori SG, Collins P, Daly C, Graham I, Jonsson B, Schenck-Gustafsson K, Tendera M. Cardiovascular diseases in women: a statement from the policy conference of the European Society of Cardiology. *Eur Heart J.* 2006;27(8):994-1005.
71. Soedamah-Muthu SS, Verberne LDM, Ding EL, Engberink MF, Geleijnse JM. Dairy consumption and incidence of hypertension. *Hypertension.* 2012;60(5):1131-1137.
72. Ralston RA, Lee JH, Truby H, Palermo CE, Walker KZ. A systematic review and meta-analysis of elevated blood pressure and consumption of dairy foods. *J Hum Hypertens.* 2012;26(1):3-13.
73. Jayedi A & Zargar MS. Dietary calcium intake and hypertension risk: a dose–response meta-analysis of prospective cohort studies. *Eur J Clin Nutr.* 2019;73(7):969-978.
74. Crichton GE, Elias MF, Dore GA, Abhayaratna WP, Robbins MA. Relations between dairy food intake and arterial stiffness: pulse wave velocity and pulse pressure. *Hypertension.* 2012;59(5):1044-1051.
75. Livingstone KM, Lovegrove JA, Cockcroft JR, Elwood PC, Pickering JE, Givens DI. Does dairy food intake predict arterial stiffness and blood pressure in men?: Evidence from the Caerphilly Prospective Study. *Hypertension.* 2013;61(1):42-47.
76. Recio-Rodriguez JI, Gomez-Marcos MA, Patino-Alonso MC, Sanchez A, Agudo-Conde C, Maderuelo-Fernandez JA, Garcia-Ortiz L. Association between fat amount of dairy products with pulse wave velocity and carotid intima-media thickness in adults. *Nutr J.* 2014;13:37.
77. Ivey KL, Hodgson JM, Kerr DA, Thompson PL, Stojceski B, Prince RL. The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial. *Nutr Metab Cardiovasc Dis.* 2015;25(1):46-51.

78. Patterson E, Larsson SC, Wolk A, Åkesson A. Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. *J Nutr.* 2012;143(1):74-79.
79. Larsson SC, Orsini N, Wolk A. Dietary calcium intake and risk of stroke: a dose-response meta-analysis. *Am J Clin Nutr.* 2013;97(5):951-957.
80. Langsetmo L, Berger C, Kreiger N, Kovacs CS, Hanley DA, Jamal SA, Whiting SJ, Genest J, Morin SN, Hodsman A et al. Calcium and vitamin D intake and mortality: results from the Canadian Multicentre Osteoporosis Study (CaMOS). *J Clin Endocrinol Metab.* 2013;98(7):3010-3018.
81. Chung M, Tang AM, Fu Z, Wang DD, Newberry SJ. Calcium intake and cardiovascular disease risk: an updated systematic review and meta-analysis. *Ann Intern Med.* 2016;165(12):856-866.
82. Asemi Z, Saneei P, Sabihi SS, Feizi A, Esmaillzadeh A. Total, dietary, and supplemental calcium intake and mortality from all-causes, cardiovascular disease, and cancer: a meta-analysis of observational studies. *Nutr Metab Cardiovasc Dis.* 2015;25(7):623-634.
83. Dehghan M, Mente A, Rangarajan S, Sheridan P, Mohan V, Iqbal R, Gupta R, Lear S, Wentzel-Viljoen E, Avezum A et al. Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (PURE): a prospective cohort study. *Lancet.* 2018;392(10161):2288-2297.
84. Michaëlsson K, Wolk A, Langenskiöld S, Basu S, Warensjö Lemming E, Melhus H, Byberg L. Milk intake and risk of mortality and fractures in women and men: cohort studies. *BMJ.* 2014;349:g6015.
85. Drouin-Chartier J-P, Brassard D, Tessier-Grenier M, Côté JA, Labonté M-È, Desroches S, Couture P, Lamarche B. Systematic review of the association between dairy product consumption and risk of cardiovascular-related clinical outcomes. *Adv Nutr.* 2016;7(6): 1026-1040.
86. Stasinopoulos LC, Zhou A, Hyppönen E. Association of supplemental calcium and dairy milk intake with all-cause and cause-specific mortality in the UK Biobank: a prospective cohort study. *Br J Nutr.* 2020;123(5):574-582.
87. Larsson SC, Crippa A, Orsini N, Wolk A, Michaelsson K. Milk consumption and mortality from all causes, cardiovascular disease, and cancer: a systematic review and meta-analysis. *Nutrients.* 2015;7(9):7749-7763.
88. Tognon G, Nilsson LM, Shungin D, Lissner L, Jansson JH, Renstrom F, Wennberg M, Winkvist A, Johansson I. Nonfermented milk and other dairy products: associations with all-cause mortality. *Am J Clin Nutr.* 2017;105(6):1502-1511.
89. Allender PS, Cutler JA, Follmann D, Cappuccio FP, Pryer J, Elliott P. Dietary calcium and blood pressure: a meta-analysis of randomized clinical trials. *Ann Intern Med.* 1996;124(9):825-831.
90. Bucher HC, Cook RJ, Guyatt GH, Lang JD, Cook DJ, Hatala R, Hunt DL. Effects of dietary calcium supplementation on blood pressure: a meta-analysis of randomized controlled trials. *JAMA.* 1996;275(13):1016-1022.
91. Griffith LE, Guyatt GH, Cook RJ, Bucher HC, Cook DJ. The influence of dietary and nondietary calcium supplementation on blood pressure*: An updated metaanalysis of randomized controlled trials. *Am J Hypertens.* 1999;12(1):84-92.

92. van Mierlo LAJ, Arends LR, Streppel MT, Zeegers MPA, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens.* 2006;20(8):571-580.
93. Chen C, Ge S, Li S, Wu L, Liu T, Li C. The effects of dietary calcium supplements alone or with vitamin D on cholesterol metabolism: a meta-analysis of randomized controlled trials. *J Cardiovasc Nurs.* 2017;32(5):496-506.
94. Dickinson HO, Mason JM, Nicolson DJ, Campbell F, Beyer FR, Cook JV, Williams B, Ford GA. Lifestyle interventions to reduce raised blood pressure: a systematic review of randomized controlled trials. *J Hypertens.* 2006;24(2):215-233.
95. Dickinson HO, Nicolson DJ, Cook JV, Campbell F, Beyer FR, Ford GA, Mason J. Calcium supplementation for the management of primary hypertension in adults. *Cochrane Database Syst Rev.* 2006; (2):Cd004639.
96. Wu L & Sun D. Effects of calcium plus vitamin D supplementation on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *J Hum Hypertens.* 2017;31(9):547-554.
97. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int.* 2007;71(5):438-441.
98. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ.* 2008;88(7638):262.
99. Mao PJ, Zhang C, Tang L, Xian YQ, Li YS, Wang WD, Zhu XH, Qiu HL, He J, Zhou YH. Effect of calcium or vitamin D supplementation on vascular outcomes: a meta-analysis of randomized controlled trials. *Int J Cardiol.* 2013;169(2):106-111.
100. Khan SU, Khan MU, Riaz H, Valavoor S, Zhao D, Vaughan L, Okunrintemi V, Riaz IB, Khan MS, Kaluski E et al. Effects of nutritional supplements and dietary interventions on cardiovascular outcomes: an umbrella review and evidence map. *Ann Intern Med.* 2019;171(3):190-198.
101. Reid IR, Bolland MJ, Grey A. Does calcium supplementation increase cardiovascular risk? *Clin Endocrinol.* 2010;73(6):689-695.
102. Reid IR, Bolland MJ, Avenell A, Grey A. Cardiovascular effects of calcium supplementation. *Osteoporos Int.* 2011;22(6):1649-1658.
103. Jenkins DJA, Spence JD, Giovannucci EL, Kim Y-i, Josse R, Vieth R, Blanco Mejia S, Viguiliouk E, Nishi S, Sahye-Pudaruth S et al. Supplemental vitamins and minerals for CVD prevention and treatment. *J Am Coll Cardiol.* 2018;71(22):2570-2584.
104. Yang C, Shi X, Xia H, Yang X, Liu H, Pan D, Sun G. The evidence and controversy between dietary calcium intake and calcium supplementation and the risk of cardiovascular disease: a systematic review and meta-analysis of cohort studies and randomized controlled trials. *J Am Coll Nutr.* 2019; doi: 10.1080/07315724.2019.1649219. [Epub ahead of print]:1-19.
105. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Control Clin Trials.* 1996;17(1):1-12.
106. Moher D, Pham B, Jones A, Cook DJ, Jadad AR, Moher M, Tugwell P, Klassen TP. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? *Lancet.* 1998;352(9128):609-613.

107. Higgins J & Green S. Cochrane handbook for systematic reviews of interventions, version 5.1.0. Higgins J, Green S, editors. London: The Cochrane Collaboration; 2011.
108. Marinou K, Christodoulides C, Antoniades C, Koutsilieris M. Wnt signaling in cardiovascular physiology. *Trends Endocrinol Metab.* 2012;23(12):628-636.
109. Joshi FR, Rajani NK, Abt M, Woodward M, Bucerius J, Mani V, Tawakol A, Kallend D, Fayad ZA, Rudd JHF. Does vascular calcification accelerate inflammation?: A substudy of the dal-PLAQUE Trial. *J Am Coll Cardiol.* 2016;67(1):69-78.
110. Albanese I, Khan K, Barratt B, Al-Kindi H, Schwertani A. Atherosclerotic calcification: Wnt is the hint. *J Am Heart Assoc.* 2018;7(4):e007356.
111. Aikawa E, Nahrendorf M, Figueiredo JL, Swirski FK, Shtatland T, Kohler RH, Jaffer FA, Aikawa M, Weissleder R. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging in vivo. *Circulation.* 2007;116(24):2841-2850.
112. New SE & Aikawa E. Molecular imaging insights into early inflammatory stages of arterial and aortic valve calcification. *Circ Res.* 2011;108(11):1381-1391.
113. Wang TJ, Larson MG, Levy D, Benjamin EJ, Kupka MJ, Manning WJ, Clouse ME, D'Agostino RB, Wilson PW, O'Donnell CJ. C-reactive protein is associated with subclinical epicardial coronary calcification in men and women: the Framingham Heart Study. *Circulation.* 2002;106(10):1189-1191.
114. Li JJ, Zhu CG, Yu B, Liu YX, Yu MY. The role of inflammation in coronary artery calcification. *Ageing Res Rev.* 2007;6(4):263-270.
115. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, Hwang SJ, Massaro JM, Larson MG, Levy D. Protein biomarkers of cardiovascular disease and mortality in the community. *J Am Heart Assoc.* 2018;7(14).
116. Blake GJ & Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res.* 2001;89(9):763-771.
117. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* 2004;351(25):2599-2610.
118. Lloyd-Jones DM, Liu K, Tian L, Greenland P. Narrative review: assessment of C-reactive protein in risk prediction for cardiovascular disease. *Ann Intern Med.* 2006;145(1):35-42.
119. Willerson JT, Wellens HJ, Cohn JN. Cardiovascular medicine. London: Springer; 2007.
120. Kampoli AM, Tousoulis D, Antoniades C, Siasos G, Stefanadis C. Biomarkers of premature atherosclerosis. *Trends Mol Med.* 2009;15(7):323-332.
121. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation.* 1998;97(20):2007-2011.
122. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342(12):836-843.
123. Libby P. Inflammation in atherosclerosis. *Nature.* 2002;420(6917):868-874.
124. Libby P, Nahrendorf M, Pittet MJ, Swirski FK. Diversity of denizens of the atherosclerotic plaque: not all monocytes are created equal. *Circulation.* 2008;117(25):3168-3170.

125. Kones R. Primary prevention of coronary heart disease: integration of new data, evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. *Drug Des Devel Ther.* 2011;5:325-380.
126. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003;107(3):499-511.
127. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med.* 2008;359(18):1897-1908.
128. Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PW. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke.* 2001;32(11):2575-2579.
129. Ford ES & Giles WH. Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. *Arterioscler Thromb Vasc Biol.* 2000;20(4):1052-1056.
130. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GDO, Pepys MB, Gudnason V. C-reactive rrotein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med.* 2004;350(14):1387-1397.
131. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. *Circulation.* 2004;109(11):1349-1353.
132. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation.* 1999;99(2):237-242.
133. Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, Polak JF, Tracy RP. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. *Circulation.* 2005;112(1):25-31.
134. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med.* 2002;347(20):1557-1565.
135. Wainstein MV, Mossmann M, Araujo GN, Gonçalves SC, Gravina GL, Sangalli M, Veadrigo F, Matte R, Reich R, Costa FG et al. Elevated serum interleukin-6 is predictive of coronary artery disease in intermediate risk overweight patients referred for coronary angiography. *Diabetol Metab Syndr.* 2017;9(1):67.
136. Puzianowska-Kuźnicka M, Owczarz M, Wieczorowska-Tobis K, Nadrowski P, Chudek J, Slusarczyk P, Skalska A, Jonas M, Franek E, Mossakowska M. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immun Ageing.* 2016;13:21-21.

137. Profumo E, Buttari B, Tosti ME, Siracusano A, Ortona E, Margutti P, Capoano R, Salvati B, Riganò R. Association of intracellular pro- and anti-inflammatory cytokines in peripheral blood with the clinical or ultrasound indications for carotid endarterectomy in patients with carotid atherosclerosis. *Clin Exp Immunol.* 2008;152(1):120-126.
138. Hou H, Wang C, Sun F, Zhao L, Dun A, Sun Z. Association of interleukin-6 gene polymorphism with coronary artery disease: an updated systematic review and cumulative meta-analysis. *Inflamm Res.* 2015;64(9):707-720.
139. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Tracy RP, Rubin SM, Harris TB, Pahor M. Inflammatory markers and cardiovascular disease (The Health, Aging and Body Composition [Health ABC] Study). *Am J Cardiol.* 2003;92(5):522-528.
140. Cesari M, Penninx BWJH, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB et al. Inflammatory markers and onset of cardiovascular events. *Circulation.* 2003;108(19):2317-2322.
141. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation.* 2000;101(15):1767-1772.
142. Su D, Li Z, Li X, Chen Y, Zhang Y, Ding D, Deng X, Xia M, Qiu J, Ling W. Association between serum interleukin-6 concentration and mortality in patients with coronary artery disease. *Mediators Inflamm.* 2013;2013:726178.
143. Caselli C, De Graaf MA, Lorenzoni V, Rovai D, Marinelli M, Del Ry S, Giannessi D, Bax JJ, Neglia D, Scholte AJ. HDL cholesterol, leptin and interleukin-6 predict high risk coronary anatomy assessed by CT angiography in patients with stable chest pain. *Atherosclerosis.* 2015;241(1):55-61.
144. Hudzik B, Szkodzinski J, Romanowski W, Danikiewicz A, Wilczek K, Lekston A, Polonski L, Zubelewicz-Szkodzinska B. Serum interleukin-6 concentration reflects the extent of asymptomatic left ventricular dysfunction and predicts progression to heart failure in patients with stable coronary artery disease. *Cytokine.* 2011;54(3):266-271.
145. Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, Gao P, Saleheen D, Rendon A, Nelson CP et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet.* 2012;379(9822):1205-1213.
146. Swerdlow DI & Holmes MV & Kuchenbaecker KB & Engmann JE & Shah T & Sofat R & Guo Y & Chung C & Peasey A & Pfister R et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. *Lancet.* 2012;379(9822):1214-1224.
147. McInnes IB, Thompson L, Giles JT, Bathon JM, Salmon JE, Beaulieu AD, Codding CE, Carlson TH, Delles C, Lee JS et al. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. *Ann Rheum Dis.* 2015;74(4):694-702.
148. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. *Circ Res.* 2016;118(1):145-156.
149. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol.* 2011;11(2):85-97.

150. Woodward L, Akoumianakis I, Antoniades C. Unravelling the adiponectin paradox: novel roles of adiponectin in the regulation of cardiovascular disease. *Br J Pharmacol.* 2017;174(22):4007-4020.
151. Ghantous CM, Azrak Z, Hanache S, Abou-Kheir W, Zeidan A. Differential role of leptin and adiponectin in cardiovascular system. *Int J Endocrinol.* 2015;2015:534320.
152. Katsiki N, Mikhailidis DP, Banach M. Leptin, cardiovascular diseases and type 2 diabetes mellitus. *Acta Pharmacol Sin.* 2018;39(7):1176-1188.
153. Kuo CH, Lin YL, Lee CJ, Wang CH, Lai YH, Liou HH, Hsu BG. Hyperleptinemia positively associated with central arterial stiffness in hemodialysis patients. *PLoS One.* 2018;13(1):e0190694.
154. Windham BG, Griswold ME, Farasat SM, Ling SM, Carlson O, Egan JM, Ferrucci L, Najjar SS. Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness. *Am J Hypertens.* 2010;23(5):501-507.
155. Sabbatini AR, Faria AP, Barbaro NR, Gordo WM, Modolo RG, Pinho C, Fontana V, Moreno H. Deregulation of adipokines related to target organ damage on resistant hypertension. *J Hum Hypertens.* 2014;28(6):388-392.
156. Gonzalez M, Lind L, Söderberg S. Leptin and endothelial function in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Atherosclerosis.* 2013;228(2):485-490.
157. Stephenson K, Tunstead J, Tsai A, Gordon R, Henderson S, Dansky HM. Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol.* 2003;23(11):2027-2033.
158. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB J.* 1999;13(10):1231-1238.
159. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci.* 2001;47(3):141-150.
160. Kalil GZ & Haynes WG. Sympathetic nervous system in obesity-related hypertension: mechanisms and clinical implications. *Hypertens Res.* 2012;35(1):4-16.
161. Liu G, Dong M, Ma S, Fu L, Xiao Y, Zhong L, Geng J. Serum leptin is associated with first-ever ischemic stroke, lesion size and stroke severity in a Chinese cohort. *Neurol Res.* 2019;41(2):125-131.
162. Khafaji HA, Bener AB, Rizk NM, Al Suwaidi J. Elevated serum leptin levels in patients with acute myocardial infarction; correlation with coronary angiographic and echocardiographic findings. *BMC Res Notes.* 2012;5:262.
163. Soderberg S, Ahren B, Jansson JH, Johnson O, Hallmans G, Asplund K, Olsson T. Leptin is associated with increased risk of myocardial infarction. *J Intern Med.* 1999;246(4):409-418.
164. Yang H, Guo W, Li J, Cao S, Zhang J, Pan J, Wang Z, Wen P, Shi X, Zhang S. Leptin concentration and risk of coronary heart disease and stroke: a systematic review and meta-analysis. *PLoS One.* 2017;12(3):e0166360.
165. Sattar N, Wannamethee G, Sarwar N, Chernova J, Lawlor DA, Kelly A, Wallace AM, Danesh J, Whincup PH. Leptin and coronary heart disease: prospective study and systematic review. *J Am Coll Cardiol.* 2009;53(2):167-175.
166. Csongradi E, Kaplar M, Nagy B, Jr., Koch CA, Juhasz A, Bajnok L, Varga Z, Seres I, Karanyi Z, Magyar MT et al. Adipokines as atherothrombotic risk factors in obese

subjects: Associations with haemostatic markers and common carotid wall thickness. *Nutr Metab Cardiovasc Dis.* 2017;27(6):571-580.
167. Ciccone M, Vettor R, Pannacciulli N, Minenna A, Bellacicco M, Rizzon P, Giorgino R, De Pergola G. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. *Int J Obes Relat Metab Disord.* 2001;25(6):805-810.
168. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257(1):79-83.
169. Ohashi K, Ouchi N, Matsuzawa Y. Anti-inflammatory and anti-atherogenic properties of adiponectin. *Biochimie.* 2012;94(10):2137-2142.
170. Ebrahimi-Mamaeghani M, Mohammadi S, Arefhosseini SR, Fallah P, Bazi Z. Adiponectin as a potential biomarker of vascular disease. *Vasc Health Risk Manag.* 2015;11:55-70.
171. Liu M & Liu F. Regulation of adiponectin multimerization, signaling and function. *Best Pract Res Clin Endocrinol Metab.* 2014;28(1):25-31.
172. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab.* 2003;285(3):E527-533.
173. Lau WB, Ohashi K, Wang Y, Ogawa H, Murohara T, Ma XL, Ouchi N. Role of adipokines in cardiovascular disease. *Circ J.* 2017;81(7):920-928.
174. Zhang H, Mo X, Hao Y, Huang J, Lu X, Cao J, Gu D. Adiponectin levels and risk of coronary heart disease: a meta-analysis of prospective studies. *Am J Med Sci.* 2013;345(6):455-461.
175. Zhang BC, Liu WJ, Che WL, Xu YW. Serum total adiponectin level and risk of cardiovascular disease in Han Chinese populations: a meta-analysis of 17 case-control studies. *Clin Endocrinol (Oxf).* 2012;77(3):370-378.
176. Gasbarrino K, Gorgui J, Nauche B, Côté R, Daskalopoulou SS. Circulating adiponectin and carotid intima-media thickness: a systematic review and meta-analysis. *Metabolism.* 2016;65(7):968-986.
177. Liberale L, Carbone F, Bertolotto M, Bonaventura A, Vecchie A, Mach F, Burger F, Pende A, Spinella G, Pane B et al. Serum adiponectin levels predict acute coronary syndrome (ACS) in patients with severe carotid stenosis. *Vascul Pharmacol.* 2018;102:37-43.
178. Stojanovic D, Buzkova P, Mukamal KJ, Heckbert SR, Psaty BM, Fink HA, Cauley JA, Wallace E, Curtis LH, Hirsch C et al. Soluble inflammatory markers and risk of incident fractures in older adults: the Cardiovascular Health Study. *J Bone Miner Res.* 2018;33(2):221-228.
179. Lencel P & Magne D. Inflammaging: the driving force in osteoporosis? *Med Hypotheses.* 2011;76(3):317-321.
180. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. *J Clin Endocrinol Metab.* 2008;93(5):1952-1958.
181. Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, Pahor M, Tylavsky FA, Cummings SR, Harris TB, Newman AB. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. *J Bone Miner Res.* 2007;22(7):1088-1095.

182. Al-Daghri NM, Aziz I, Yakout S, Aljohani NJ, Al-Saleh Y, Amer OE, Sheshah E, Younis GZ, Al-Badr FB. Inflammation as a contributing factor among postmenopausal Saudi women with osteoporosis. *Medicine.* 2017;96(4):e5780.
183. Barbour KE, Lui LY, Ensrud KE, Hillier TA, LeBlanc ES, Ing SW, Hochberg MC, Cauley JA. Inflammatory markers and risk of hip fracture in older white women: the study of osteoporotic fractures. *J Bone Miner Res.* 2014;29(9):2057-2064.
184. Ganesan K, Teklehaimanot S, Tran T-H, Asuncion M, Norris K. Relationship of C-reactive protein and bone mineral density in community-dwelling elderly females. *J Natl Med Assoc.* 2005;97(3):329-333.
185. Cervellati C, Bonaccorsi G, Bergamini CM, Fila E, Greco P, Valacchi G, Massari L, Gonelli A, Tisato V. Association between circulatory levels of adipokines and bone mineral density in postmenopausal women. *Menopause.* 2016;23(9):984-992.
186. Sponholtz TR, Zhang X, Fontes JD, Meigs JB, Cupples LA, Kiel DP, Hannan MT, McLean RR. Association between inflammatory biomarkers and bone mineral density in a community-based cohort of men and women. *Arthritis Care Res.* 2014;66(8):1233-1240.
187. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. *J Clin Endocrinol Metab.* 2001;86(5):2032-2042.
188. Barbour KE, Boudreau R, Danielson ME, Youk AO, Wactawski-Wende J, Greep NC, LaCroix AZ, Jackson RD, Wallace RB, Bauer DC et al. Inflammatory markers and the risk of hip fracture: the Women's Health Initiative. *J Bone Miner Res.* 2012;27(5):1167-1176.
189. Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, Kim GS. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int.* 2005;16(10):1263-1271.
190. Pasco JA, Kotowicz MA, Henry MJ, Nicholson GC, Spilsbury HJ, Box JD, Schneider HG. High-sensitivity C-reactive protein and fracture risk in elderly women. *JAMA.* 2006;296(11):1349-1355.
191. Nakamura K, Saito T, Kobayashi R, Oshiki R, Oyama M, Nishiwaki T, Nashimoto M, Tsuchiya Y. C-reactive protein predicts incident fracture in community-dwelling elderly Japanese women: the Muramatsu study. *Osteoporos Int.* 2011;22(7):2145-2150.
192. Schett G, Kiechl S, Weger S, Pederiva A, Mayr A, Petrangeli M, Oberhollenzer F, Lorenzini R, Redlich K, Axmann R et al. High-sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. *Arch Intern Med.* 2006;166(22):2495-2501.
193. Ishii S, Cauley JA, Greendale GA, Crandall CJ, Danielson ME, Ouchi Y, Karlamangla AS. C-reactive protein, bone strength, and nine-year fracture risk: data from the Study of Women's Health Across the Nation (SWAN). *J Bone Miner Res.* 2013;28(7):1688-1698.
194. Dahl K, Ahmed LA, Joakimsen RM, Jorgensen L, Eggen AE, Eriksen EF, Bjornerem A. High-sensitivity C-reactive protein is an independent risk factor for non-vertebral fractures in women and men: The Tromso Study. *Bone.* 2015;72:65-70.
195. Chen XX & Yang T. Roles of leptin in bone metabolism and bone diseases. *J Bone Miner Metab.* 2015;33(5):474-485.
196. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell.* 2000;100(2):197-207.

197. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell.* 2002;111(3):305-317.
198. Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. *Cell metabolism.* 2006;4(5):341-348.
199. Schwetz V, Pieber T, Obermayer-Pietsch B. The endocrine role of the skeleton: background and clinical evidence. *Eur J Endocrinol.* 2012;166(6):959-967.
200. Cornish J, Callon KE, Bava U, Lin C, Naot D, Hill BL, Grey AB, Broom N, Myers DE, Nicholson GC et al. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *J Endocrinol.* 2002;175(2):405-415.
201. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology.* 1999;140(4):1630-1638.
202. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab.* 2011;301(4):E567-584.
203. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* 2004;34(3):376-383.
204. Haam JH, Kim YS, Kim MJ, Koo HS, Kim HY, Kim HJ, Park KH, Joo NS, Park KC. A cross-sectional study of the association between adipokine levels and bone mineral density according to obesity and menopausal status in Korean women. *J Bone Miner Metab.* 2017;35(6):642-648.
205. Zhang H, Xie H, Zhao Q, Xie GQ, Wu XP, Liao EY, Luo XH. Relationships between serum adiponectin, apelin, leptin, resistin, visfatin levels and bone mineral density, and bone biochemical markers in post-menopausal Chinese women. *J Endocrinol Invest.* 2010;33(10):707-711.
206. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, Kanaya AM, Harris TB, Cauley JA, Health ABCS. The effects of adiponectin and leptin on changes in bone mineral density. *Osteoporos Int.* 2012;23(6):1699-1710.
207. Dennison EM, Syddall HE, Fall CH, Javaid MK, Arden NK, Phillips DI, Cooper C. Plasma leptin concentration and change in bone density among elderly men and women: the Hertfordshire Cohort Study. *Calcif Tissue Int.* 2004;74(5):401-406.
208. Weiss LA, Barrett-Connor E, von Muhlen D, Clark P. Leptin predicts BMD and bone resorption in older women but not older men: the Rancho Bernardo study. *J Bone Miner Res.* 2006;21(5):758-764.
209. Roux C, Arabi A, Porcher R, Garnero P. Serum leptin as a determinant of bone resorption in healthy postmenopausal women. *Bone.* 2003;33(5):847-852.
210. Biver E, Salliot C, Combescure C, Gossec L, Hardouin P, Legroux-Gerot I, Cortet B. Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-Analysis. *J Clin Endocrinol Metab.* 2011;96(9):2703-2713.
211. Schett G, Kiechl S, Bonora E, Redlich K, Woloszczuk W, Oberhollenzer F, Jocher J, Dorizzi R, Muggeo M, Smolen J et al. Serum leptin level and the risk of nontraumatic fracture. *Am J Med.* 2004;117(12):952-956.
212. Pepe J, Cipriani C, Cilli M, Colangelo L, Minisola S. Adipokines and bone metabolism: an interplay to untangle. *J Endocrinol Invest.* 2016;39(11):1359-1361.

213. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, Kanaya AM, Harris TB, Bauer DC, Cauley JA. Adipokines and the risk of fracture in older adults. *J Bone Miner Res.* 2011;26(7):1568-1576.
214. Tanna N, Patel K, Moore AE, Dulnoan D, Edwards S, Hampson G. The relationship between circulating adiponectin, leptin and vaspin with bone mineral density (BMD), arterial calcification and stiffness: a cross-sectional study in post-menopausal women. *J Endocrinol Invest.* 2017;40(12):1345-1353.
215. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Yano S, Sugimoto T. Relationships between serum adiponectin levels versus bone mineral density, bone metabolic markers, and vertebral fractures in type 2 diabetes mellitus. *Eur J Endocrinol.* 2009;160(2):265-273.
216. Kanazawa I, Yamaguchi T, Sugimoto T. Baseline serum total adiponectin level is positively associated with changes in bone mineral density after 1-year treatment of type 2 diabetes mellitus. *Metabolism.* 2010;59(9):1252-1256.
217. Sodi R, Hazell MJ, Durham BH, Rees C, Ranganath LR, Fraser WD. The circulating concentration and ratio of total and high molecular weight adiponectin in post-menopausal women with and without osteoporosis and its association with body mass index and biochemical markers of bone metabolism. *Clin Biochem.* 2009;42(13-14):1375-1380.
218. Kontogianni MD, Dafni UG, Routsias JG, Skopouli FN. Blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in perimenopausal women. *J Bone Miner Res.* 2004;19(4):546-551.
219. Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR. Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res.* 2005;20(11):1912-1920.
220. Vassalle C & Mazzone A. Bone loss and vascular calcification: a bi-directional interplay? *Vascul Pharmacol.* 2016;86:77-86.
221. Wiklund P, Nordstrom A, Jansson JH, Weinehall L, Nordstrom P. Low bone mineral density is associated with increased risk for myocardial infarction in men and women. *Osteoporos Int.* 2012;23(3):963-970.
222. den Uyl D, Nurmohamed MT, van Tuyl LH, Raterman HG, Lems WF. (Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk; a systematic review of the association between cardiovascular disease and osteoporosis. *Arthritis Res Ther.* 2011;13(1):R5.
223. Värri M, Tuomainen T-P, Honkanen R, Rikkonen T, Niskanen L, Kröger H, Tuppurainen MT. Carotid intima-media thickness and calcification in relation to bone mineral density in postmenopausal women — The OSTPRE-BBA study. *Maturitas.* 2014;78(4):304-309.
224. Simon SP, Fodor D, Muntean L, Poanta L, Cristea P, Rednic S. Bone mineral density, vertebral fractures and body mass index in postmenopausal women with abdominal aortic calcification. *Endocr Res.* 2014;39(1):1-6.
225. Schulz E, Arfai K, Liu X, Sayre J, Gilsanz V. Aortic calcification and the risk of osteoporosis and fractures. *J Clin Endocrinol Metab.* 2004;89(9):4246-4253.
226. Hak AE, Pols HA, van Hemert AM, Hofman A, Witteman JC. Progression of aortic calcification is associated with metacarpal bone loss during menopause: a population-based longitudinal study. *Arterioscler Thromb Vasc Biol.* 2000;20(8):1926-1931.

227. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. *Calcif Tissue Int.* 2001;68(5):271-276.
228. Farhat GN, Cauley JA, Matthews KA, Newman AB, Johnston J, Mackey R, Edmundowicz D, Sutton-Tyrrell K. Volumetric BMD and vascular calcification in middle-aged women: the Study of Women's Health Across the Nation. *J Bone Miner Res.* 2006;21(12):1839-1846.
229. Hyder JA, Allison MA, Wong N, Papa A, Lang TF, Sirlin C, Gapstur SM, Ouyang P, Carr JJ, Criqui MH. Association of coronary artery and aortic calcium with lumbar bone density: the MESA Abdominal Aortic Calcium Study. *Am J Epidemiol.* 2009;169(2):186-194.
230. Bagger YZ, Tanko LB, Alexandersen P, Qin G, Christiansen C. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. *J Intern Med.* 2006;259(6):598-605.
231. Liu D, Chen L, Dong S, Peng Z, Yang H, Chen Y, Li L, Zhou H, Zhou R. Bone mass density and bone metabolism marker are associated with progression of carotid and cardiac calcified plaque in Chinese elderly population. *Osteoporos Int.* 2019;30(9):1807-1815.
232. Zhou R, Zhou H, Cui M, Chen L, Xu J. The association between aortic calcification and fracture risk in postmenopausal women in China: the prospective Chongqing osteoporosis study. *PLoS One.* 2014;9(5):e93882-e93882.
233. Lewis JR, Eggermont CJ, Schousboe JT, Lim WH, Wong G, Khoo B, Sim M, Yu M, Ueland T, Bollerslev J et al. Association between abdominal aortic calcification, bone mineral density and fracture in older women. *J Bone Miner Res.* 2019;34(11):2052-2060.
234. Szulc P. Vascular calcification and fracture risk. *Clin Cases Miner Bone Metab.* 2015;12(2):139-141.
235. Szulc P, Kiel DP, Delmas PD. Calcifications in the abdominal aorta predict fractures in men: MINOS study. *J Bone Miner Res.* 2008;23(1):95-102.
236. Szulc P, Blackwell T, Schousboe JT, Bauer DC, Cawthon P, Lane NE, Cummings SR, Orwoll ES, Black DM, Ensrud KE. High hip fracture risk in men with severe aortic calcification: MrOS study. *J Bone Miner Res.* 2014;29(4):968-975.
237. Liu G-Y, Liang Q-H, Cui R-R, Liu Y, Wu S-S, Shan P-F, Yuan L-Q, Liao E-Y. Leptin promotes the osteoblastic differentiation of vascular smooth muscle cells from female mice by increasing RANKL expression. *Endocrinology.* 2014;155(2):558-567.
238. Zhan J-K, Wang Y-J, Wang Y, Wang S, Tan P, Huang W, Liu Y-S. The mammalian target of rapamycin signalling pathway is involved in osteoblastic differentiation of vascular smooth muscle cells. *Can J Cardiol.* 2014;30(5):568-575.
239. Zhan J-K, Wang Y-J, Wang Y, Tang Z-Y, Tan P, Huang W, Liu Y-S. Adiponectin attenuates the osteoblastic differentiation of vascular smooth muscle cells through the AMPK/mTOR pathway. *Exp Cell Res.* 2014;323(2):352-358.
240. Lu Y, Bian Y, Wang Y, Bai R, Wang J, Xiao C. Globular adiponectin reduces vascular calcification via inhibition of ER-stress-mediated smooth muscle cell apoptosis. *Int J Clin Exp Pathol.* 2015;8(3):2545-2554.
241. Farhat GN & Cauley JA. The link between osteoporosis and cardiovascular disease. *Clin Cases Miner Bone Metab.* 2008;5(1):19-34.

242. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *J Clin Invest.* 2005;115(12):3318-3325.
243. Wang TJ. Vitamin D and cardiovascular disease. *Annu Rev Med.* 2016;67:261-272.
244. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. *Circulation.* 2008;117(4):503-511.
245. Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. *Atherosclerosis.* 2009;205(1):255-260.
246. Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). *Am J Cardiol.* 2008;102(11):1540-1544.
247. Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J, Raggi P. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: results from NHANES 2001 to 2004. *Arterioscler Thromb Vasc Biol.* 2008;28(6):1179-1185.
248. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med.* 2008;168(11):1174-1180.
249. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension.* 2007;49(5):1063-1069.
250. Manson JE, Cook NR, Lee I-M, Christen W, Bassuk SS, Mora S, Gibson H, Gordon D, Copeland T, D'Agostino D et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med.* 2018;380(1):33-44.
251. Wu-Wong JR, Nakane M, Ma J, Ruan X, Kroeger PE. Effects of Vitamin D analogs on gene expression profiling in human coronary artery smooth muscle cells. *Atherosclerosis.* 2006;186(1):20-28.
252. Wakasugi M, Noguchi T, Inoue M, Kazama Y, Tawata M, Kanemaru Y, Onaya T. Vitamin D3 stimulates the production of prostacyclin by vascular smooth muscle cells. *Prostaglandins.* 1991;42(2):127-136.
253. de Francisco AL. Secondary hyperparathyroidism: review of the disease and its treatment. *Clin Ther.* 2004;26(12):1976-1993.
254. Laroche M, Pecourneau V, Blain H, Breuil V, Chapurlat R, Cortet B, Sutter B, Degboe Y. Osteoporosis and ischemic cardiovascular disease. *Joint Bone Spine.* 2017;84(4):427-432.
255. Lampropoulos CE, Papaioannou I, D'Cruz DP. Osteoporosis – a risk factor for cardiovascular disease? *Nat Rev Rheumatol.* 2012;8(10):587-598.
256. van Ballegooijen AJ, Reinders I, Visser M, Brouwer IA. Parathyroid hormone and cardiovascular disease events: a systematic review and meta-analysis of prospective studies. *Am Heart J.* 2013;165(5):655-664.e655.
257. Hagstrom E, Hellman P, Larsson TE, Ingelsson E, Berglund L, Sundstrom J, Melhus H, Held C, Lind L, Michaelsson K et al. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation.* 2009;119(21):2765-2771.
258. Melhus H, Michaelsson K, Larsson SC. Serum parathyroid hormone and risk of coronary artery disease: exploring causality using Mendelian randomization. *J Clin Endocrinol Metab.* 2019;104(11):5595-5600.

259. Lv F, Cai X, Yang W, Gao L, Chen L, Wu J, Ji L. Denosumab or romosozumab therapy and risk of cardiovascular events in patients with primary osteoporosis: systematic review and meta- analysis. *Bone.* 2020;130:115121.
260. Schett G & Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat Rev Rheumatol.* 2012;8(11):656-664.
261. Mezquita-Raya P, de la Higuera M, Garcia DF, Alonso G, Ruiz-Requena ME, de Dios Luna J, Escobar-Jimenez F, Munoz-Torres M. The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women. *Osteoporos Int.* 2005;16(11):1368-1374.
262. Kiechl S, Werner P, Knoflach M, Furtner M, Willeit J, Schett G. The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. *Expert Rev Cardiovasc Ther.* 2006;4(6):801-811.
263. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL et al. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev.* 1998;12(9):1260-1268.
264. Venuraju SM, Yerramasu A, Corder R, Lahiri A. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J Am Coll Cardiol.* 2010;55(19):2049-2061.
265. Schoppet M, Sattler AM, Schaefer JR, Herzum M, Maisch B, Hofbauer LC. Increased osteoprotegerin serum levels in men with coronary artery disease. *J Clin Endocrinol Metab.* 2003;88(3):1024-1028.
266. George SJ. Wnt pathway. *Arterioscler Thromb Vasc Biol.* 2008;28(3):400-402.
267. Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lowik CW, Reeve J. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J.* 2005;19(13):1842-1844.
268. Ardawi M-SM, Rouzi AA, Al-Sibiani SA, Al-Senani NS, Qari MH, Mousa SA. High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the center of excellence for osteoporosis research study. *J Bone Miner Res.* 2012;27(12):2592-2602.
269. Wijenayaka AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS One.* 2011;6(10):e25900.
270. Durosier C, van Lierop A, Ferrari S, Chevalley T, Papapoulos S, Rizzoli R. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. *J Clin Endocrinol Metab.* 2013;98(9):3873-3883.
271. Mödder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, Melton LJ, 3rd, Khosla S. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. *J Bone Miner Res.* 2011;26(2):373-379.
272. Costa AG, Walker MD, Zhang CA, Cremers S, Dworakowski E, McMahon DJ, Liu G, Bilezikian JP. Circulating sclerostin levels and markers of bone turnover in Chinese-American and White women. *J Clin Endocrinol Metab.* 2013;98(12):4736-4743.
273. He J, Zhang H, Wang C, Zhang Z, Yue H, Hu W, Gu J, Fu W, Hu Y, Li M et al. Associations of serum sclerostin and polymorphisms in the SOST gene with bone mineral density and markers of bone metabolism in postmenopausal Chinese women. *J Clin Endocrinol Metab.* 2014;99(4):E665-E673.

274. Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int.* 2013;24(2):489-494.
275. Dawson-Hughes B, Harris SS, Ceglia L, Palermo NJ. Serum sclerostin levels vary with season. *J Clin Endocrinol Metab.* 2014;99(1):E149-E152.
276. Arasu A, Cawthon PM, Lui L-Y, Do TP, Arora PS, Cauley JA, Ensrud KE, Cummings SR, The Study of Osteoporotic Fractures Research G. Serum sclerostin and risk of hip fracture in older Caucasian women. *J Clin Endocrinol Metab.* 2012;97(6):2027-2032.
277. Zheng J, Maerz W, Gergei I, Kleber M, Drechsler C, Wanner C, Brandenburg V, Reppe S, Gautvik KM, Medina-Gomez C et al. Mendelian randomization analysis reveals a causal influence of circulating sclerostin levels on bone mineral density and fractures. *J Bone Miner Res.* 2019;34(10):1824-1836.
278. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, Langdahl BL, Reginster J-Y, Zanchetta JR, Wasserman SM et al. Romosozumab in postmenopausal women with low bone mineral density. *N Engl J Med.* 2014;370(5):412-420.
279. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, Maddox J, Fan M, Meisner PD, Grauer A. Romosozumab or alendronate for fracture prevention in women with osteoporosis. *N Engl J Med.* 2017;377(15):1417-1427.
280. Krishna SM, Seto SW, Jose RJ, Li J, Morton SK, Biros E, Wang Y, Nsengiyumva V, Lindeman JH, Loots GG et al. Wnt signaling pathway inhibitor sclerostin inhibits angiotensin II-induced aortic aneurysm and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2017;37(3):553-566.
281. Brandenburg VM, Kramann R, Koos R, Krüger T, Schurgers L, Mühlenbruch G, Hübner S, Gladziwa U, Drechsler C, Ketteler M. Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. *BMC Nephrol.* 2013;14(1):219.
282. Shao J-S, Cheng S-L, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA. Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J Clin Invest.* 2005;115(5):1210-1220.
283. Zhu D, Mackenzie NC, Millán JL, Farquharson C, MacRae VE. The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PLoS One.* 2011;6(5):e19595.
284. Novo-Rodríguez C, García-Fontana B, Luna-Del Castillo JDD, Andújar-Vera F, Ávila-Rubio V, García-Fontana C, Morales-Santana S, Rozas-Moreno P, Muñoz-Torres M. Circulating levels of sclerostin are associated with cardiovascular mortality. *PLoS One.* 2018;13(6):e0199504.
285. Hampson G, Edwards S, Conroy S, Blake GM, Fogelman I, Frost ML. The relationship between inhibitors of the Wnt signalling pathway (Dickkopf-1(DKK1) and sclerostin), bone mineral density, vascular calcification and arterial stiffness in post-menopausal women. *Bone.* 2013;56(1):42-47.
286. Gaudio A, Fiore V, Rapisarda R, Sidoti MH, Xourafa A, Catalano A, Tringali G, Zanoli L, Signorelli SS, Fiore CE. Sclerostin is a possible candidate marker of arterial stiffness: Results from a cohort study in Catania. *Mol Med Rep.* 2017;15(5):3420-3424.

287. Gaudio A, Privitera F, Pulvirenti I, Canzonieri E, Rapisarda R, Fiore CE. The relationship between inhibitors of the Wnt signalling pathway (sclerostin and Dickkopf-1) and carotid intima-media thickness in postmenopausal women with type 2 diabetes mellitus. *Diab Vasc Dis Res.* 2014;11(1):48-52.
288. Nguyen-Yamamoto L, Tanaka KI, St-Arnaud R, Goltzman D. Vitamin D-regulated osteocytic sclerostin and BMP2 modulate uremic extraskeletal calcification. *JCI Insight.* 2019;4(13).
289. Reid IR, Mason B, Horne A, Ames R, Reid HE, Bava U, Bolland MJ, Gamble GD. Randomized controlled trial of calcium in healthy older women. *Am J Med.* 2006;119(9):777-785.
290. Reid IR, Ames R, Mason B, Reid HE, Bacon CJ, Bolland MJ, Gamble GD, Grey A, Horne A. Randomized controlled trial of calcium supplementation in healthy, nonosteoporotic, older men. *Arch Intern Med.* 2008;168(20):2276-2282.
291. Gagnon C, Daly RM, Carpentier A, Lu ZX, Shore-Lorenti C, Sikaris K, Jean S, Ebeling PR. Effects of combined calcium and vitamin D supplementation on insulin secretion, insulin sensitivity and beta-cell function in multi-ethnic vitamin D-deficient adults at risk for type 2 diabetes: a pilot randomized, placebo-controlled trial. *PLoS One.* 2014;9(10):e109607.
292. Tabesh M, Azadbakht L, Faghihimani E, Tabesh M, Esmaillzadeh A. Calcium-vitamin D cosupplementation influences circulating inflammatory biomarkers and adipocytokines in vitamin D-insufficient diabetics: a randomized controlled clinical trial. *J Clin Endocrinol Metab.* 2014;99(12):E2485-E2493.
293. Dundar U, Kavuncu V, Ciftci IH, Evcik D, Solak O, Cakir T. The effect of risedronate treatment on serum cytokines in postmenopausal osteoporosis: a 6-month randomized and controlled study. *J Bone Miner Metab.* 2009;27(4):464-470.
294. Grey A, Gamble G, Ames R, Horne A, Mason B, Reid IR. Calcium supplementation does not affect CRP levels in postmenopausal women-a randomized controlled trial. *Osteoporos Int.* 2006;17(8):1141-1145.
295. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets higher in dairy foods and dietary protein support bone health during diet- and exercise-induced weight loss in overweight and obese premenopausal women. *J Clin Endocrinol Metab.* 2012;97(1):251-260.
296. Neyestani TR, Nikooyeh B, Alavi-Majd H, Shariatzadeh N, Kalayi A, Tayebinejad N, Heravifard S, Salekzamani S, Zahedirad M. Improvement of vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. *J Clin Endocrinol Metab.* 2012;97(6):2005-2011.
297. Ulven SM, Holven KB, Gil A, Rangel-Huerta OD. Milk and dairy product consumption and inflammatory biomarkers: an updated systematic review of randomized clinical trials. *Adv Nutr.* 2019;10(Suppl 2):S239-S250.
298. Benatar JR, Sidhu K, Stewart RA. Effects of high and low fat dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. *PLoS One.* 2013;8(10):e76480.
299. Serra MC, Beavers KM, Beavers DP, Willoughby DS. Effects of 28 days of dairy or soy ingestion on skeletal markers of inflammation and proteolysis in post-menopausal women. *Nutr Health.* 2012;21(2):117-130.

300. Jafarnejad S, Djafarian K, Fazeli MR, Yekaninejad MS, Rostamian A, Keshavarz SA. Effects of a multispecies probiotic supplement on bone health in osteopenic postmenopausal women: a randomized, double-blind, controlled trial. *J Am Coll Nutr.* 2017;36(7):497-506.
301. Ohlsson C, Engdahl C, Fåk F, Andersson A, Windahl SH, Farman HH, Movérare-Skrtic S, Islander U, Sjögren K. Probiotics protect mice from ovariectomy-induced cortical bone loss. *PLoS One.* 2014;9(3):e92368.
302. Plessas S, Bosnea L, Alexopoulos A, Bezirtzoglou E. Potential effects of probiotics in cheese and yogurt production: A review. *Eng Life Sci.* 2012;12(4):433-440.
303. Biró G, Hulshof KF, Ovesen L, Amorim Cruz JA, Group E. Selection of methodology to assess food intake. *Eur J Clin Nutr.* 2002;56:25-32.
304. Boushey CJ. Nutritional epidemiology: dietary assessment methods. In: Weaver C, Heaney RP, editors. Calcium in human health. Totowa: Humana Press; 2006. p. 39-63.
305. Palaniappan U, Cue RI, Payette H, Gray-Donald K. Implications of day-to-day variability on measurements of usual food and nutrient intakes. *J Nutr.* 2003;133(1):232-235.
306. Nusser SM, Carriquiry AL, Dodd KW, Fuller WA. A semiparametric transformation approach to estimating usual daily intake distributions. *J AM Stat Assoc.* 1996;91(436):1440-1449.
307. Willett W. Nature of variation in diet. In: Willett W, editor. Nutritional epidemiology. New York: Oxford University Press; 2012.
308. Baranowski T. 24-hour recall and diet record methods. In: Willett W, editor. Nutritional epidemiology. New York: Oxford University Press; 2012.
309. Serra-Majem L, Frost Andersen L, Henrique-Sanchez P, Doreste-Alonso J, Sanchez-Villegas A, Ortiz-Andrelluchi A, Negri E, La Vecchia C. Evaluating the quality of dietary intake validation studies. *Br J Nutr.* 2009;102 Suppl 1:S3-9.
310. Willett W. Food frequency methods. In: Willett W, editor. Nutritional epidemiology. Oxford: Oxford University Press; 2012. p. 70-95.
311. Poliquin S, Joseph L, Gray-Donald K. Calcium and vitamin D intakes in an adult Canadian population. *Can J Diet Pract Res.* 2009;70(1):21-27.
312. Willett W & Lenart E. Reproducibility and validity of food frequency questionnaires. In: Willett W, editor. Nutritional epidemiology. Oxford: Oxford University Press; 2012. p. 91-141.
313. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA.* 2005;293(18):2257-2264.
314. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: evidence from a comparative metaanalysis of randomized controlled trials. *J Clin Endocrinol Metab.* 2007;92(4):1415-1423.
315. Zhu K, Prince RL, Bone, Calcium. Calcium and bone. *Clin Biochem.* 2012;45(12):936-942.
316. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, Bassford T, Beresford SA, Black HR, Blanchette P et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354(7):669-683.
317. Papaioannou A, Morin S, Cheung AM, Atkinson S, Brown JP, Feldman S, Hanley DA, Hodsman A, Jamal SA, Kaiser SM et al. 2010 clinical practice guidelines for the

diagnosis and management of osteoporosis in Canada: summary. *CMAJ.* 2010;182(17):1864-1873.
318. Foote JA, Murphy SP, Wilkens LR, Hankin JH, Henderson BE, Kolonel LN. Factors associated with dietary supplement use among healthy adults of five ethnicities: the multiethnic cohort study. *Am J Epidemiol.* 2003;157:888-897.
319. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J.* 2007;28(12):1462-1536.
320. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension.* 2001;37(5):1236-1241.
321. Kanis JA on behalf of the World Health Organization Scientific Group. Assessment of osteoporosis at the primary healthcare level. Technical Report. 2008. Internet: http://www.shef.ac.uk/FRAX. Accessed October 13, 2013.
322. Bauer DC. Calcium supplements and fracture prevention. *N Engl J Med.* 2014;370(4):387-388.
323. BC Dairy Foundation. Calcium Calculator. 2007. Internet: http://bcdairyfoundation.ca/interactive/calcium-calculator. Accessed October 15, 2013.
324. Health Canada Bureau of Nutritional Sciences. Using the Nutrition Facts Table - % Daily Value. Ottawa, Canada: Health Canada; 2011.
325. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc Sport.* 2000;71(2 Suppl):S114-120.
326. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* 2003;35(8):1381-1395.
327. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol.* 1985;122(1):51-65.
328. International Physical Activity Questionnaire. Guidelines for the data processing and analysis of the International Physical Activity Questionnaire - Short and long forms. 2005. Internet: http://www.ipaq.ki.se. Accessed July 21, 2015.
329. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *Am J Clin Nutr.* 2003;77(5):1171-1178.
330. Health Canada. Canadian guidelines for body weight classification in adults. 2003. Internet: https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/healthy-weights/canadian-guidelines-body-weight-classification-adults/questions-answers-public.html. Accessed October 10, 2013.
331. Fitness Canada. Canadian Standard Test of Fitness (CSTF): Operations Manual. Ottawa: Fitness and Amateur Sport, Government of Canada; 1986.
332. Daskalopoulou SS, Khan NA, Quinn RR, Ruzicka M, McKay DW, Hackam DG, Rabkin SW, Rabi DM, Gilbert RE, Padwal RS et al. The 2012 Canadian Hypertension Education Program recommendations for the management of hypertension: blood pressure

measurement, diagnosis, assessment of risk, and therapy. *Can J Cardiol.* 2012;28(3):270-287.
333. AtCor Medical. Software operator's guide: pulse wave analysis system SCOR-Px. Sydney, Australia: AtCor Medical; 2006.
334. Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, Kaiser DR, Thuillez C. Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *Am J Hypertens.* 2002;15(5):445-452.
335. Gorgui J, Doonan RJ, Gomez YH, Kwong C, Daskalopoulou SS. Carotid endarterectomy improves peripheral but not central arterial stiffness. *Eur J Vasc Endovasc Surg.* 2013;45(6):548-553.
336. Doonan RJ, Mutter A, Egiziano G, Gomez Y-H, Daskalopoulou SS. Differences in arterial stiffness at rest and after acute exercise between young men and women. *Hypertens Res.* 2013;36(3):226-231.
337. Yu A, Giannone T, Scheffler P, Doonan RJ, Egiziano G, Gomez Y-H, Papaioannou TG, Daskalopoulou SS. The effect of oral Contraceptive pills and the natural menstrual cYCLe on arterial stiffness and hemodynamICs (CYCLIC). *J Hypertens.* 2014;32(1):100-107.
338. Doonan RJ, Scheffler P, Yu A, Egiziano G, Mutter A, Bacon S, Carli F, Daskalopoulos ME, Daskalopoulou SS. Altered arterial stiffness and subendocardial viability ratio in young healthy light smokers after acute exercise. *PLoS One.* 2011;6(10):e26151.
339. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499-502.
340. Wason JM, Stecher L, Mander AP. Correcting for multiple-testing in multi-arm trials: is it necessary and is it done? *Trials.* 2014;15:364.
341. Salvi P. Pulse waves: how vascular hemodynamics affects blood pressure. Milan: Springer; 2012.
342. Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J.* 2010;31(19):2338-2350.
343. Daskalopoulou SS, Rabi DM, Zarnke KB, Dasgupta K, Nerenberg K, Cloutier L, Gelfer M, Lamarre-Cliche M, Milot A, Bolli P et al. The 2015 Canadian Hypertension Education Program recommendations for blood pressure measurement, diagnosis, assessment of risk, prevention, and treatment of hypertension. *Can J Cardiol.* 2015;31(5):549-568.
344. Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, Anderson FH, Cooper C, Francis RM, Donaldson C et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet.* 2005;365(9471):1621-1628.
345. Prince RL, Devine A, Dhaliwal SS, Dick IM. Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double-blind, placebo-controlled trial in elderly women. *Arch Intern Med.* 2006;166(8):869-875.
346. Burckhardt P. Potential negative cardiovascular effects of calcium supplements. *Osteoporos Int.* 2011;22(6):1645-1647.

347. Rubin MR, Rundek T, McMahon DJ, Lee HS, Sacco RL, Silverberg SJ. Carotid artery plaque thickness is associated with increased serum calcium levels: the Northern Manhattan study. *Atherosclerosis.* 2007;194(2):426-432.
348. Foley RN, Collins AJ, Ishani A, Kalra PA. Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J.* 2008;156(3):556-563.
349. Bolland MJ, Wang TKM, van Pelt NC, Horne AM, Mason BH, Ames RW, Grey AB, Ruygrok PN, Gamble GD, Reid IR. Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. *J Bone Miner Res.* 2010;25(3):505-512.
350. Reid IR, Schooler BA, Hannan SF, Ibbertson HK. The acute biochemical effects of four proprietary calcium preparations. *Aust N Z J Med.* 1986;16(2):193-197.
351. Burt MG, Mangelsdorf BL, Petersons CJ, Srivastava D. Acute effect of calcium citrate on serum calcium and cardiovascular function. *J Bone Miner Res.* 2013;28(2):412-418.
352. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol.* 2010;55(13):1318-1327.
353. Mitchell GF, Hwang S-J, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events the Framingham Heart Study. *Circulation.* 2010;121(4):505-511.
354. Recio-Rodriguez JI, Gomez-Marcos MA, Patino-Alonso MC, Sanchez A, Agudo-Conde C, Maderuelo-Fernandez JA, Garcia-Ortiz L. Association between fat amount of dairy products with pulse wave velocity and carotid intima-media thickness in adults. *Nutr J.* 2014;13:37.
355. Yaron M, Roach V, Izkhakov E, Ish-Shalom M, Sack J, Sofer Y, Azzam I, Ray A, Stern N, Tordjman KM. Effects of a typical acute oral calcium load on arterial properties and endothelial function in healthy subjects. *Eur J Clin Nutr.* 2014;68(5):608-612.
356. Reid IR, Mason B, Horne A, Ames R, Clearwater J, Bava U, Orr-Walker B, Wu F, Evans MC, Gamble GD. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. *Am J Med.* 2002;112(5):343-347.
357. Chai W, Cooney RV, Franke AA, Bostick RM. Effects of calcium and vitamin D supplementation on blood pressure and serum lipids and carotenoids: a randomized, double-blind, placebo-controlled, clinical trial. *Ann Epidemiol.* 2013;23(9):564-570.
358. Karandish M, Shockravi S, Jalali MT, Haghighizadeh MH. Effect of calcium supplementation on lipid profile in overweight or obese Iranian women: a double-blind randomized clinical trial. *Eur J Clin Nutr.* 2009;63(2):268-272.
359. Rajpathak SN, Xue X, Wassertheil-Smoller S, Van Horn L, Robinson JG, Liu S, Allison M, Martin LW, Ho GY, Rohan TE. Effect of 5 y of calcium plus vitamin D supplementation on change in circulating lipids: results from the Women's Health Initiative. *Am J Clin Nutr.* 2010;91(4):894-899.
360. Reid IR, Ames R, Mason B, Bolland MJ, Bacon CJ, Reid HE, Kyle C, Gamble GD, Grey A, Horne A. Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: a randomized controlled trial. *Am J Clin Nutr.* 2010;91(1):131-139.

361. Lorenzen JK, Nielsen S, Holst JJ, Tetens I, Rehfeld JF, Astrup A. Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. *Am J Clin Nutr.* 2007;85(3):678-687.
362. Reid IR, Mason B, Horne A, Ames R, Clearwater J, Bava U, Orr-Walker B, Wu F, Evans MC, Gamble GD. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. *Am J Med.* 2002;112(5):343-347.
363. Ditscheid B, Keller S, Jahreis G. Cholesterol metabolism is affected by calcium phosphate supplementation in humans. *J Nutr.* 2005;135(7):1678-1682.
364. Bostick RM, Fosdick L, Grandits GA, Grambsch P, Gross M, Louis TA. Effect of calcium supplementation on serum cholesterol and blood pressure. A randomized, double-blind, placebo-controlled, clinical trial. *Arch Fam Med.* 2000;9(1):31-38; discussion 39.
365. Fleischman AI, Yacowitz H, Hayton T, Bierenbaum ML. Effects of dietary calcium upon lipid metabolism in mature male rats fed beef tallow. *J Nutr.* 1966;88(3):255-260.
366. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr.* 2007;85(6):1586-1591.
367. Weaver CM, Alexander DD, Boushey CJ, Dawson-Hughes B, Lappe JM, LeBoff MS, Liu S, Looker AC, Wallace TC, Wang DD. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. *Osteoporos Int.* 2016;27(1):367-376.
368. Peterlik M & Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest.* 2005;35(5):290-304.
369. McCarron DA. Hypertension and cardiovascular disease. In: Weaver C, Heaney RP, editors. Calcium in human health. Totowa: Humana Press; 2006. p. 421-429.
370. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington, DC: National Academy of Sciences; 2011.
371. Canadian Food Inspection Agency. Foods to which vitamins, mineral, nutrients and amino acids may or must be added. 2014. Internet: http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/nutrient-content/reference-information/eng/1389908857542/1389908896254?chap=1. Accessed January 3, 2020.
372. Health Canada. Interim Marketing Authorization to permit the optional addition of vitamins and mineral nutrients to plant-based beverages. 2013. Internet: http://www.hc-sc.gc.ca/fn-an/legislation/ima-amp/plant_based_beverages-boissons_vegetales-eng.php. Accessed February 8, 2017.
373. Health Canada. Canadian Nutrient File 2010b version. 2012. Internet: http://webprod3.hc-sc.gc.ca/cnf-fce/. Accessed February 2, 2016.
374. Health Canada. Canadian Community Health Survey, Cycle 2.2, Nutrition (2004): a guide to accessing and interpreting the data. Ottawa: Office of Nutrition Policy and Promotion, Health Products and Food Branch, Health Canada; 2006. Available from: http://publications.gc.ca/collections/Collection/H164-20-2006E.pdf.
375. Gibson RS. Measuring food consumption of individuals. In: Gibson RS, editor. Principles of nutritional assessment. New York: Oxford University Press; 2005. p. 41-64.

376. Serra-Majem L, Andersen LF, Henrique-Sanchez P, Doreste-Alonso J, Sanchez-Villegas A, Ortiz-Andrelluchi A, Negri E., La Vecchia C. Evaluating the quality of dietary intake validation studies. *Br J Nutr.* 2009;102:S3-S9.
377. Hacker-Thompson A, Robertson TP, Sellmeyer DE. Validation of two food frequency questionnaires for dietary calcium assessment. *J Am Diet Assoc.* 2009;109(7):1237-1240.
378. Sebring NG, Denkinger BI, Menzie CM, Yanoff LB, Parikh SJ, Yanovski JA. Validation of three food frequency questionnaires to assess dietary calcium intake in adults. *J Am Diet Assoc.* 2007;107(5):752-759.
379. Satalic Z, Colicbaric I, Cecic I, Keser I. Short food frequency questionnaire can discriminate inadequate and adequate calcium intake in Croatian postmenopausal women. *Nutr Res.* 2007;27(9):542-547.
380. Clover E, Miller M, Bannerman E, Magarey A. Relative validation of a short food frequency questionnaire to assess calcium intake in older adults. *Aust N Z J Public Health.* 2007;31(5):450-458.
381. Magkos F, Manios Y, Babaroutsi E, Sidossis L. Development and validation of a food frequency questionnaire for assessing dietary calcium intake in the general population. *Osteoporos Int.* 2006;17(2):304-312.
382. Montomoli M, Gonnelli S, Giacchi M, Mattei R, Cuda C, Rossi S, Gennari C. Validation of a food frequency questionnaire for nutritional calcium intake assessment in Italian women. *Eur J Clin Nutr.* 2002;56(1):21-30.
383. Magarey A, Baulderstone L, Yaxley A, Markow K, Miller M. Evaluation of tools used to measure calcium and/or dairy consumption in adults. *Public Health Nutr.* 2015;18(07):1225-1236.
384. Cade J, Thompson R, Burley V, Warm D. Development, validation and utilisation of food-frequency questionnaires - a review. *Public Health Nutr.* 2001;5(4):567-587.
385. Langsetmo L, Berger C, Kreiger N, Kovacs CS, Hanley DA, Jamal SA, Whiting SJ, Genest J, Morin SN, Hodsman A et al. Calcium and vitamin D intake and mortality: results from the Canadian Multicentre Osteoporosis Study (CaMOS). *J Clin Endocrinol Metab.* 2013;98(7):3010-3018.
386. Ong AM, Weiler HA, Wall M, Haddad R, Gorgui J, Daskalopoulou SS, Goltzman D, Morin SN. Feasibility of a clinical trial to assess the effect of dietary calcium v. supplemental calcium on vascular and bone markers in healthy postmenopausal women. *Br J Nutr.* 2016;116(1):104-114.
387. Shatenstein B & Payette H. Evaluation of the relative validity of the short diet questionnaire for assessing usual consumption frequencies of selected nutrients and foods. *Nutrients.* 2015;7(8):6362-6374.
388. Hung A, Hamidi M, Riazantseva E, Thompson L, Tile L, Tomlinson G, Stewart B, Cheung AM. Validation of a calcium assessment tool in postmenopausal Canadian women. *Maturitas.* 2011;69(2):168-172.
389. United States Department of Agriculture. How much do you eat? 2006. Internet: https://www.fns.usda.gov/sites/default/files/ne_ppt.pdf. Accessed October 13, 2019.
390. Ghasemi A & Zahediasl S. Normality tests for statistical analysis: a guide for non-statisticians. *Int J Endocrinol Metab.* 2012;10(2):486-489.
391. Fleiss JL & Cohen J. The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educ Psychol Meas.* 1973;33(3):613-619.

392. Altman DG & Bland JM. Statistics notes: quartiles, quintiles, centiles, and other quantiles. *BMJ.* 1994;309(6960).
393. Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, Wei L, Grubb DA, Bolton-Smith C. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr.* 2003;6(3):313-321.
394. Mielke Jr PW & Berry KJ. A note on Cohen's weighted kappa coefficient of agreement with linear weights. *Stat Methodol.* 2009;6(5):439-446.
395. Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991.
396. Bland JM & Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1(8476):307-310.
397. Bland JM & Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res.* 1999;8(2):135-160.
398. Hebert JR, Clemow L, Pbert L, Ockene IS, Ockene JK. Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. *Int J Epidemiol.* 1995;24(2):389-398.
399. Hebert JR, Hurley TG, Steck SE, Miller DR, Tabung FK, Peterson KE, Kushi LH, Frongillo EA. Considering the value of dietary assessment data in informing nutrition-related health policy. *Adv Nutr.* 2014;5(4):447-455.
400. Lyu L-C, Hankin JH, Liu LQ, Wilkens LR, Lee JH, Goodman MT, Kolonel LN. Telephone vs face-to-face interviews for quantitative food frequency assessment. *J Am Diet Assoc.* 1998;98(1):44-48.
401. Schaffer DM, Coates AO, Caan BJ, Slattery ML, Potter JD. Performance of a shortened telephone-administered version of a quantitative food frequency questionnaire. *Ann Epidemiol.* 1997;7(7):463-471.
402. Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM. Food-frequency questionnaires: a review of their design, validation and utilisation. *Nutr Res Rev.* 2004;17(1):5-22.
403. Musgrave KO, Giambalvo L, Leclerc HL, Cook RA, Rosen CJ. Validation of a quantitative food frequency questionnaire for rapid assessment of dietary calcium intake. *J Am Diet Assoc.* 1989;89(10):1484-1488.
404. Pasco JA, Sanders KM, Henry MJ, Nicholson GC, Seeman E, Kotowicz MA. Calcium intakes among Australian women: Geelong Osteoporosis Study. *Aust N Z J Med.* 2000;30(1):21-27.
405. Plawecki KL, Evans EM, Mojtahedi MC, McAuley E, Chapman-Novakofski K. Assessing calcium intake in postmenopausal women. *Prev Chronic Dis.* 2009;6(4):A124.
406. Cummings SR, Block G, McHenry K, Baron RB. Evaluation of two food frequency methods of measuring dietary calcium intake. *Am J Epidemiol.* 1987;126(5):796-802.
407. Matthys C, Pynaert I, Roe M, Fairweather-Tait SJ, Heath AL, De Henauw S. Validity and reproducibility of a computerised tool for assessing the iron, calcium and vitamin C intake of Belgian women. *Eur J Clin Nutr.* 2004;58(9):1297-1305.
408. Pritchard JM, Seechurn T, Atkinson SA. A food frequency questionnaire for the assessment of calcium, vitamin D and vitamin K: a pilot validation study. *Nutrients.* 2010;2(8):805-819.

409. Wirfält AK, Jeffery RW, Elmer PJ. Comparison of food frequency questionnaires: the reduced Block and Willett questionnaires differ in ranking on nutrient intakes. *Am J Epidemiol.* 1998;148(12):1148-1156.
410. Baranowski T. 24-hour dietary recall and food record methods. In: Willett W, editor. Nutritional epidemiology. Oxford: Oxford Press; 2013. p. 49-69.
411. Wirfalt AKE, Jeffery RW, Elmer PJ. Comparison of food frequency questionnaires: the reduced Block and Willett questionnaires differ in ranking on nutrient intakes. *Am J Epidemiol.* 1998;148(12):1148-1156.
412. Hoy M & Goldman J. Calcium intake of the U.S. population: What We Eat in America, NHANES 2009-2010. Food Surveys Research Group Dietary Data Brief No. 13. 2014. Internet: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/DBrief/13_calcium_intake_0910.pdf. Accessed November 12, 2016.
413. Wilson P & Horwath C. Validation of a short food frequency questionnaire for assessment of dietary calcium intake in women. *Eur J Clin Nutr.* 1996;50(4):220-228.
414. Michael Nelson & Bingham SA. Assessment of food consumption and nutrient intake. In: Margetts B, Nelson M, editors. Design concepts in nutritional epidemiology. Oxford: Oxford University Press; 1997. p. 123-169.
415. Bian S, Hu J, Zhang K, Wang Y, Yu M, Ma J. Dairy product consumption and risk of hip fracture: a systematic review and meta-analysis. *BMC Public Health.* 2018;18(1):165.
416. Johnell O & Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int.* 2006;17(12):1726-1733.
417. International Osteoporosis Foundation. Facts and Statistics. 2017. Internet: https://www.iofbonehealth.org/facts-statistics. Accessed Nov 20, 2017.
418. Reid IR. Menopause. In: Rosen CJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. Iowa: Wiley-Blackwell; 2013. p. 165-170.
419. Balk EM, Adam GP, Langberg VN, Earley A, Clark P, Ebeling PR, Mithal A, Rizzoli R, Zerbini CAF, Pierroz DD et al. Global dietary calcium intake among adults: a systematic review. *Osteoporos Int.* 2017;28(12):3315-3324.
420. Canadian Food Inspection Agency. Specific nutrient content claim requirements - Vitamin and mineral nutrient claims. 2018. Internet: http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/nutrient-content/specific-claim-requirements/eng/1389907770176/1389907817577?chap=13. Accessed Accessed January 3, 2020.
421. Moschonis G & Manios Y. Skeletal site-dependent response of bone mineral density and quantitative ultrasound parameters following a 12-month dietary intervention using dairy products fortified with calcium and vitamin D: the Postmenopausal Health Study. *Br J Nutr.* 2006;96(06):1140-1148.
422. Sahni S, Tucker KL, Kiel DP, Quach L, Casey VA, Hannan MT. Milk and yogurt consumption are linked with higher bone mineral density but not with hip fracture: the Framingham Offspring Study. *Arch Osteoporos.* 2013;8(1):119.
423. Heaney RP. Dairy and bone health. *J Am Coll Nutr.* 2009;28(Suppl 1):82S-90S.
424. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, Kanis JA, Orav EJ, Staehelin HB, Kiel DP, Burckhardt P, Henschkowski J, Spiegelman D et al. Milk intake and risk of hip fracture in men and women: A meta-analysis of prospective cohort studies. *J Bone Miner Res.* 2011;26(4):833-839.

425. Kanis JA, Johansson H, Oden A, De Laet C, Johnell O, Eisman JA, McCloskey E, Mellstrom D, Pols H, Reeve J et al. A meta-analysis of milk intake and fracture risk: low utility for case finding. *Osteoporos Int.* 2005;16(7):799-804.
426. Bolland MJ, Leung W, Tai V, Bastin S, Gamble GD, Grey A, Reid IR. Calcium intake and risk of fracture: systematic review. *BMJ.* 2015;351:h4580.
427. Heller KJ. Probiotic bacteria in fermented foods: product characteristics and starter organisms. *Am J Clin Nutr.* 2001;73(2):374s-379s.
428. Östman B, Michaëlsson K, Helmersson J, Byberg L, Gedeborg R, Melhus H, Basu S. Oxidative stress and bone mineral density in elderly men: antioxidant activity of alpha-tocopherol. *Free Radic Biol Med.* 2009;47(5):668-673.
429. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. *Immunol Rev.* 2005;208(1):207-227.
430. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6(7):e1000097.
431. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfleger B, Khaltaev N. Assessment of fracture risk. *Osteoporos Int.* 2005;16(6):581-589.
432. Stone KL, Seeley DG, Lui L-Y, Cauley JA, Ensrud K, Browner WS, Nevitt MC, Cummings SR. BMD at multiple sites and risk of fracture of multiple types: long-term results from the study of osteoporotic fractures. *J Bone Miner Res.* 2003;18(11):1947-1954.
433. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, Eisman JA, Fujiwara S, Kroger H, Mellstrom D et al. Predictive value of BMD for hip and other fractures. *J Bone Miner Res.* 2005;20(7):1185-1194.
434. Leslie WD, Tsang JF, Caetano PA, Lix LM. Effectiveness of bone density measurement for predicting osteoporotic fractures in clinical practice. *J Clin Endocrinol Metab.* 2007;92(1):77-81.
435. Cummings SR, Browner W, Cummings SR, Black DM, Nevitt MC, Browner W, Genant HK, Cauley J, Ensrud K, Scott J et al. Bone density at various sites for prediction of hip fractures. *Lancet.* 1993;341(8837):72-75.
436. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: technical report series 843. Geneva: World Health Organization; 1994.
437. Kanis JA, Melton III LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res.* 1994;9(8):1137-1141.
438. World Health Organization. (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO Study Group [meeting held in Rome from 22 to 25 June 1992]. WHO technical report series; 843. p. 1-129.
439. Bruyere O, Collette J, Delmas P, Rouillon A, Roux C, Seidel L, Richy F, Reginster J-Y. Interest of biochemical markers of bone turnover for long-term prediction of new vertebral fracture in postmenopausal osteoporotic women. *Maturitas.* 2003;44(4):259-265.
440. Chapurlat RD, Garnero P, Brárt G, Meunier PJ, Delmas PD. Serum type I collagen breakdown product (serum CTX) predicts hip fracture risk in elderly women: The EPIDOS study. *Bone.* 2000;27(2):283-286.

441. Garnero P & Delmas PD. Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. *J Musculoskelet Neuronal Interact.* 2004;4(1):50-63.
442. Grados F, Brazier M, Kamel S, Mathieu M, Hurtebize N, Maamer M, Garabédian Ml, Sebert J-L, Fardellone P. Prediction of bone mass density variation by bone remodeling markers in postmenopausal women with vitamin D insufficiency treated with calcium and vitamin D supplementation. *J Clin Endocrinol Metab.* 2003;88(11):5175-5179.
443. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *J Bone Miner Res.* 2000;15(8):1526-1536.
444. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The use of biochemical markers of bone turnover in osteoporosis. *Osteoporos Int.* 2000;11:2-17.
445. Hlaing TT & Compston JE. Biochemical markers of bone turnover – uses and limitations. *Ann Clin Biochem.* 2014;51(2):189-202.
446. Higgins JPT SJ, Savović J, Page MJ, Hróbjartsson A, Boutron I, Reeves B, Eldridge S. A revised tool for assessing risk of bias in randomized trials. *Cochrane Database Syst Rev.* 2016;10(Suppl 1):29-31.
447. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle–Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. 2000. Internet: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed August 21, 2018.
448. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, deBeer H et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol.* 2011;64(4):383-394.
449. DerSimonian R & Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177-188.
450. Hardy RJ & Thompson SG. Detecting and describing heterogeneity in meta-analysis. *Stat Med.* 1998;17(8):841-856.
451. Higgins JPT & Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002;21(11):1539-1558.
452. Michaëlsson K, Wolk A, Lemming EW, Melhus H, Byberg L. Intake of milk or fermented milk combined with fruit and vegetable consumption in relation to hip fracture rates: a cohort study of Swedish women. *J Bone Miner Res.* 2018;33(3):449-457.
453. Sahni S, Mangano KM, Tucker KL, Kiel DP, Casey VA, Hannan MT. Protective association of milk intake on the risk of hip fracture: Results from the Framingham original cohort. *J Bone Miner Res.* 2014;29(8):1756-1762.
454. Sahni S, Mangano KM, Kiel DP, Tucker KL, Hannan MT. Dairy intake is protective against bone loss in older vitamin D supplement users: the Framingham Study. *J Nutr.* 2017;147(4):645-652.
455. Johnson J, Mistry V, Vukovich M, Hogie-Lorenzen T, Hollis B, Specker B. Bioavailability of vitamin D from fortified process cheese and effects on vitamin D status in the elderly. *J Dairy Sci.* 2005;88(7):2295-2301.
456. Keramat A, Patwardhan B, Larijani B, Chopra A, Mithal A, Chakravarty D, Adibi H, Khosravi A. The assessment of osteoporosis risk factors in Iranian women compared with Indian women. *BMC Musculoskelet Disord.* 2008;9(28).

457. Tu MY, Chen HL, Tung YT, Kao CC, Hu FC, Chen CM. Short-term effects of kefir-fermented milk consumption on bone mineral density and bone metabolism in a randomized clinical trial of osteoporotic patients. *PLoS One.* 2015;10(12):e0144231.
458. Jha RM, Mithal A, Malhotra N, Brown EM. Pilot case-control investigation of risk factors for hip fractures in the urban Indian population. *BMC Musculoskelet Disord.* 2010;11:49.
459. Feskanich D, Meyer HE, Fung TT, Bischoff-Ferrari HA, Willett WC. Milk and other dairy foods and risk of hip fracture in men and women. *Osteoporos Int.* 2018;29(2):385-396.
460. Tavani A, Negri E, La Vecchia C. Calcium, dairy products, and the risk of hip fracture in women in northern Italy. *Epidemiology.* 1995;6(5):554-557.
461. Grgurevic A, Gledovic Z, Vujasinovic-Stupar N. Factors associated with postmenopausal osteoporosis: a case-control study of Belgrade women. *Women & Health.* 2010;50(5):475-490.
462. Heaney R, Rafferty K, Dowell M. Effect of yogurt on a urinary marker of bone resorption in postmenopausal women. *J Am Diet Assoc.* 2002;102(11):1672-1674.
463. Bonjour JP, Benoit V, Rousseau B, Souberbielle JC. Consumption of vitamin D- and calcium-fortified soft white cheese lowers the biochemical marker of bone resorption TRAP 5b in postmenopausal women at moderate risk of osteoporosis fracture. *J Nutr.* 2012;142(4):698-703.
464. Feart C, Lorrain S, Ginder Coupez V, Samieri C, Letenneur L, Paineau D, Barberger-Gateau P. Adherence to a Mediterranean diet and risk of fractures in French older persons. *Osteoporos Int.* 2013;24(12):3031-3041.
465. Panahi S, Fernandez MA, Marette A, Tremblay A. Yogurt, diet quality and lifestyle factors. *Eur J Clin Nutr.* 2016;71:573.
466. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA.* 2001;285(6):785-795.
467. Sayón-Orea C, Bes-Rastrollo M, Martí A, Pimenta AM, Martín-Calvo N, Martínez-González MA. Association between yogurt consumption and the risk of metabolic syndrome over 6 years in the SUN study. *BMC Public Health.* 2015;15(1):170.
468. Martinez-Gonzalez MA, Sayon-Orea C, Ruiz-Canela M, de la Fuente C, Gea A, Bes-Rastrollo M. Yogurt consumption, weight change and risk of overweight/obesity: The SUN cohort study. *Nutr Metab Cardiovasc Dis.* 2014;24(11):1189-1196.
469. Zhu K & Prince RL. Lifestyle and osteoporosis. *Curr Osteoporos Rep.* 2015;13(1):52-59.
470. Laird E, Molloy AM, McNulty H, Ward M, McCarroll K, Hoey L, Hughes CF, Cunningham C, Strain JJ, Casey MC. Greater yogurt consumption is associated with increased bone mineral density and physical function in older adults. *Osteoporos Int.* 2017;28(8):2409-2419.
471. Biver E, Durosier-Izart C, Merminod F, Chevalley T, van Rietbergen B, Ferrari SL, Rizzoli R. Fermented dairy products consumption is associated with attenuated cortical bone loss independently of total calcium, protein, and energy intakes in healthy postmenopausal women. *Osteoporos Int.* 2018;29(8):1771-1782.
472. Hsu E & Pacifici R. From osteoimmunology to osteomicrobiology: how the microbiota and the immune system regulate bone. *Calcif Tissue Int.* 2018;102(5):512-521.

473. Boyce BF, Schwarz EM, Xing L. Osteoclast precursors: cytokine-stimulated immunomodulators of inflammatory bone disease. *Curr Opin Rheumatol.* 2006;18(4):427-432.
474. Rizzoli R & Biver E. Effects of fermented milk products on bone. *Calcif Tissue Int.* 2018;102(4):489-500.
475. Collins FL, Irwin R, Bierhalter H, Schepper J, Britton RA, Parameswaran N, McCabe LR, van Wijnen AE. Lactobacillus reuteri 6475 increases bone density in intact females only under an inflammatory setting. *PLoS One.* 2016;11(4).
476. Britton RA, Irwin R, Quach D, Schaefer L, Zhang J, Lee T, Parameswaran N, McCabe LR. Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell Physiol.* 2014;229(11):1822-1830.
477. Nilsson AG, Sundh D, Bäckhed F, Lorentzon M. Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med.* 2018;284(3):307-317.
478. Ivey KL, Lewis JR, Hodgson JM, Zhu K, Dhaliwal SS, Thompson PL, Prince RL. Association between yogurt, milk, and cheese consumption and common carotid artery intima-media thickness and cardiovascular disease risk factors in elderly women. *Am J Clin Nutr.* 2011;94(1):234-239.
479. Pei R, DiMarco DM, Putt KK, Martin DA, Gu Q, Chitchumroonchokchai C, White HM, Scarlett CO, Bruno RS, Bolling BW. Low-fat yogurt consumption reduces biomarkers of chronic inflammation and inhibits markers of endotoxin exposure in healthy premenopausal women: a randomised controlled trial. *Br J Nutr.* 2017;118(12):1043-1051.
480. Dugan CE, Aguilar D, Park YK, Lee JY, Fernandez ML. Dairy consumption lowers systemic inflammation and liver enzymes in typically low-dairy consumers with clinical characteristics of metabolic syndrome. *J Am Coll Nutr.* 2016;35(3):255-261.
481. Nestel PJ, Mellett N, Pally S, Wong G, Barlow CK, Croft K, Mori TA, Meikle PJ. Effects of low-fat or full-fat fermented and non-fermented dairy foods on selected cardiovascular biomarkers in overweight adults. *Br J Nutr.* 2013;110(12):2242-2249.
482. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am J Clin Nutr.* 2010;91(1):16-22.
483. Stancliffe RA, Thorpe T, Zemel MB. Dairy attentuates oxidative and inflammatory stress in metabolic syndrome. *Am J Clin Nutr.* 2011;94(2):422-430.
484. Health Canada. Canadian Nutrient File 2010b version. Internet: http://webprod3.hc-sc.gc.ca/cnf-fce/. Accessed February 2, 2016.
485. Health Canada. Evidence review for dietary guidance: technical report 2015. Ottawa: Health Canada; 2016.
486. Tai V, Leung W, Grey A, Reid IR, Bolland MJ. Calcium intake and bone mineral density: systematic review and meta-analysis. *BMJ.* 2015;351:h4183.
487. Heaney RP. Calcium, dairy products and osteoporosis. *J Am Coll Nutr.* 2000;19(Suppl 2):83s-99s.
488. Knox EG. Ischaemic-heart-disease mortality and dietary intake of calcium. *Lancet.* 1973;1(7818):1465-1467.

489. Umesawa M, Iso H, Date C, Yamamoto A, Toyoshima H, Watanabe Y, Kikuchi S, Koizumi A, Kondo T, Inaba Y et al. Dietary intake of calcium in relation to mortality from cardiovascular disease: the JACC Study. *Stroke.* 2006;37(1):20-26.
490. Bauer DC. The calcium supplement controversy: Now what? *J Bone Miner Res.* 2014;29(3):531-533.
491. Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med.* 1999;340(2):115-126.
492. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong JC, Khan H, Baena CP, Prabhakaran D, Hoshen MB et al. Vitamin D and risk of cause specific death systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ.* 2014;348.
493. Lupton JR, Faridi KF, Martin SS, Sharma S, Kulkarni K, Jones SR, Michos ED. Deficient serum 25-hydroxyvitamin D is associated with an atherogenic lipid profile: The Very Large Database of Lipids (VLDL-3) study. *J Clin Lipidol.* 2016;10(1):72-81.e71.
494. Wang L, Song Y, Manson JE, Pilz S, März W, Michaëlsson K, Lundqvist A, Jassal SK, Barrett-Connor E, Zhang C et al. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes.* 2012;5(6):819-829.
495. National Institutes of Health & National Heart, Lung, and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults — the evidence report. *Obes Res.* 1998;6 Suppl 2:51s-209s.
496. Lau DCW, Douketis JD, Morrison KM, Hramiak IM, Sharma AM, Ur E, Obesity Canada Clinical Practice, Guidelines Expert Panel. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. *CMAJ.* 2007;176(8):S1-S13.
497. Ong AM, Weiler HA, Wall M, Goltzman D, Whiting SJ, Daskalopoulou SS, Morin SN. A 51-item calcium-focused food frequency questionnaire is a reliable tool to assess dietary calcium intake in postmenopausal women. *Nutr Res.* 2017;43:33-42.
498. Morin SN, Ong AM, Weiler HA, Wall M, Berger C, Das S, Cheung AM, Rahme E, Goltzman D, Daskalopoulou SS. A randomized clinical trial on the effect of dietary calcium intake as compared to calcium supplement on vascular health in postmenopausal women. *J Bone Miner Res.* 2019;32(Suppl 1):S26.
499. Bell ML, Fiero M, Horton NJ, Hsu C-H. Handling missing data in RCTs; a review of the top medical journals. *BMC Med Res Methodol.* 2014;14(1):118.
500. DeSouza CM, Legedza AT, Sankoh AJ. An overview of practical approaches for handling missing data in clinical trials. *J Biopharm Stat.* 2009;19(6):1055-1073.
501. Puntus T, Schneider B, Meran J, Peterlik M, Kudlacek S. Influence of age and gender on associations of body mass index with bone mineral density, bone turnover markers and circulating calcium-regulating and bone-active sex hormones. *Bone.* 2011;49(4):824-829.
502. Adami S, Bianchi G, Brandi ML, Giannini S, Ortolani S, DiMunno O, Frediani B, Rossini M, group ObotBs. Determinants of bone turnover markers in healthy premenopausal women. *Calcif Tissue Int.* 2008;82(5):341-347.
503. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. *J Clin Endocrinol Metab.* 2010;95(4):1991-1997.

504. Schautz B, Later W, Heller M, Peters A, Muller MJ, Bosy-Westphal A. Impact of age on leptin and adiponectin independent of adiposity. *Br J Nutr.* 2012;108(2):363-370.
505. Zhu S, Patel KV, Bandinelli S, Ferrucci L, Guralnik JM. Predictors of interleukin-6 elevation in older adults. *J Am Geriatr Soc.* 2009;57(9):1672-1677.
506. Gottsater M, Ostling G, Persson M, Engstrom G, Melander O, Nilsson PM. Non-hemodynamic predictors of arterial stiffness after 17 years of follow-up: the Malmo Diet and Cancer study. *J Hypertens.* 2015;33(5):957-965.
507. Ma H, Lin H, Hu Y, Li X, He W, Jin X, Gao J, Zhao N, Liu Z, Gao X. Serum 25-hydroxyvitamin D levels are associated with carotid atherosclerosis in normotensive and euglycemic Chinese postmenopausal women: the Shanghai Changfeng study. *BMC Cardiovasc Disord.* 2014;14:197-197.
508. Elhakeem A, Cooper R, Whincup P, Brage S, Kuh D, Hardy R. Physical activity, sedentary time, and cardiovascular disease biomarkers at age 60 to 64 years. *J Am Heart Assoc.* 2018;7(16):e007459.
509. Shimegi S, Yanagita M, Okano H, Yamada M, Fukui H, Fukumura Y, Ibuki Y, Kojima I. Physical exercise increases bone mineral density in postmenopausal women. *Endocr J.* 1994;41(1):49-56.
510. Bouassida A, Latiri I, Bouassida S, Zalleg D, Zaouali M, Feki Y, Gharbi N, Zbidi A, Tabka Z. Parathyroid hormone and physical exercise: a brief review. *J Sports Sci Med.* 2006;5(3):367-374.
511. Sahni S, Mangano KM, Tucker KL, Kiel DP, Casey VA, Hannan MT. Protective association of milk intake on the risk of hip fracture: results from the Framingham Original Cohort. *J Bone Miner Res.* 2014;29(8):1756-1762.
512. Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw.* 2015;67(1):1-48.
513. Kuznetsova A BP, Christensen RHB (2017). lmerTest package: tests in linear mixed effects models. *J Stat Softw.* 2017;82(13):1–26.
514. Baeksgaard L, Andersen KP, Hyldstrup L. Calcium and vitamin D supplementation increases spinal BMD in healthy, postmenopausal women. *Osteoporos Int.* 1998;8(3):255-260.
515. Bonnick S, Broy S, Kaiser F, Teutsch C, Rosenberg E, DeLucca P, Melton M. Treatment with alendronate plus calcium, alendronate alone, or calcium alone for postmenopausal low bone mineral density. *Curr Med Res Opin.* 2007;23(6):1341-1349.
516. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ. Vitamin D3 and calcium to prevent hip fractures in elderly women. *N Engl J Med.* 1992;327(23):1637-1642.
517. Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, Garnero P, Meunier PJ. Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. *Osteoporos Int.* 2002;13(3):257-264.
518. Harwood RH, Sahota O, Gaynor K, Masud T, Hosking DJ. A randomised, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: The Nottingham Neck of Femur (NONOF) Study. *Age Ageing.* 2004;33(1):45-51.
519. LaCroix AZ, Kotchen J, Anderson G, Brzyski R, Cauley JA, Cummings SR, Gass M, Johnson KC, Ko M, Larson J et al. Calcium plus vitamin D supplementation and

mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. *J Gerontol A Biol Sci Med Sci.* 2009;64(5):559-567.
520. Porthouse J, Cockayne S, King C, Saxon L, Steele E, Aspray T, Baverstock M, Birks Y, Dumville J, Francis R et al. Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. *BMJ.* 2005;330(7498):1003.
521. Riggs BL, O'Fallon WM, Muhs J, O'Connor MK, Kumar R, Melton LJ, 3rd. Long-term effects of calcium supplementation on serum parathyroid hormone level, bone turnover, and bone loss in elderly women. *J Bone Miner Res.* 1998;13(2):168-174.
522. Salovaara K, Tuppurainen M, Karkkainen M, Rikkonen T, Sandini L, Sirola J, Honkanen R, Alhava E, Kroger H. Effect of vitamin D(3) and calcium on fracture risk in 65- to 71-year-old women: a population-based 3-year randomized, controlled trial — the OSTPRE-FPS. *J Bone Miner Res.* 2010;25(7):1487-1495.
523. Bonjour JP, Brandolini-Bunlon M, Boirie Y, Morel-Laporte F, Braesco V, Bertiere MC, Souberbielle JC. Inhibition of bone turnover by milk intake in postmenopausal women. *Br J Nutr.* 2008;100(4):866-874.
524. Fardellone P, Brazier M, Kamel S, Gueris J, Graulet AM, Lienard J, Sebert JL. Biochemical effects of calcium supplementation in postmenopausal women: influence of dietary calcium intake. *Am J Clin Nutr.* 1998;67(6):1273-1278.
525. Tenta R, Moschonis G, Koutsilieris M, Manios Y. Calcium and vitamin D supplementation through fortified dairy products counterbalances seasonal variations of bone metabolism indices: the Postmenopausal Health Study. *Eur J Nutr.* 2011;50(5):341-349.
526. Karlamangla AS, Burnett-Bowie SM, Crandall CJ. Bone health during the menopause transition and beyond. *Obstet Gynecol Clin North Am.* 2018;45(4):695-708.
527. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Higgins JP, Lennon L, Eiriksdottir G et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med.* 2008;5(4):e78.
528. Tsai JP. The association of serum leptin levels with metabolic diseases. *Ci Ji Yi Xue Za Zhi.* 2017;29(4):192-196.
529. Gannagé-Yared MH, Azoury M, Mansour I, Baddoura R, Halaby G, Naaman R. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. *J Endocrinol Invest.* 2003;26(8):748-753.
530. Fakhrawi D, Beeson W, Nakhoul R, Darnell T, Cordero-MacIntyre Z. Dairy calcium intake and relationship to bone mineral density (BMD), bone mineral content (BMC) and leptin in post-menopausal women. *Eur J Nutr Food Saf.* 2017;7(4):244-253.
531. Fonolla-Joya J, Reyes-Garcia R, Garcia-Martin A, Lopez-Huertas E, Munoz-Torres M. Daily intake of milk enriched with n-3 fatty acids, oleic acid, and calcium improves metabolic and bone biomarkers in postmenopausal women. *J Am Coll Nutr.* 2016;35(6):529-536.
532. Phillips-Eakley AK, McKenney-Drake ML, Bahls M, Newcomer SC, Radcliffe JS, Wastney ME, Van Alstine WG, Jackson G, Alloosh M, Martin BR et al. Effect of high-calcium diet on coronary artery disease in Ossabaw miniature swine with metabolic syndrome. *J Am Heart Assoc.* 2015;4(8):e001620-e001620.

533. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J.* 2000;14(9):1132-1138.
534. Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, Hinders S. Calcium intake and body weight. *J Clin Endocrinol Metab.* 2000;85(12):4635-4638.
535. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. *Science.* 1984;224(4656):1392-1398.
536. Dougkas A, Reynolds CK, Givens ID, Elwood PC, Minihane AM. Associations between dairy consumption and body weight: a review of the evidence and underlying mechanisms. *Nutr Res Rev.* 2011;24(1):72-95.
537. Shalileh M, Shidfar F, Haghani H, Eghtesadi S, Heydari I. The influence of calcium supplement on body composition, weight loss and insulin resistance in obese adults receiving low calorie diet. *J Res Med Sci.* 2010;15(4):191-201.
538. Barr SI. Increased dairy product or calcium intake: is body weight or composition affected in humans? *J Nutr.* 2003;133(1):245s-248s.
539. Shalileh M, Shidfar F, Haghani H, Eghtesadi S, Heydari I. The influence of calcium supplement on body composition, weight loss and insulin resistance in obese adults receiving low calorie diet. *J Res Med Sci.* 2010;15(4):191-201.
540. Li P, Fan C, Lu Y, Qi K. Effects of calcium supplementation on body weight: a meta-analysis. *Am J Clin Nutr.* 2016;104(5):1263-1273.
541. Stevens J, Truesdale KP, McClain JE, Cai J. The definition of weight maintenance. *Int J Obes.* 2006;30(3):391-399.
542. Booth AO, Huggins CE, Wattanapenpaiboon N, Nowson CA. Effect of increasing dietary calcium through supplements and dairy food on body weight and body composition: a meta-analysis of randomised controlled trials. *Br J Nutr.* 2015;114(7):1013-1025.
543. Tajik N, Keshavarz SA, Masoudkabir F, Djalali M, Sadrzadeh-Yeganeh HH, Eshraghian MR, Chamary M, Ahmadivand Z, Yazdani T, Javanbakht MH. Effect of diet-induced weight loss on inflammatory cytokines in obese women. *J Endocrinol Invest.* 2013;36(4):211-215.
544. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, Mergl R, Kirkby KC, Faßhauer M, Stumvoll M et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS One.* 2015;10(3):e0121971.
545. Barr SI, McCarron DA, Heaney RP, Dawson-Hughes B, Berga SL, Stern JS, Oparil S. Effects of increased consumption of fluid milk on energy and nutrient intake, body weight, and cardiovascular risk factors in healthy older adults. *J Am Diet Assoc.* 2000;100(7):810-817.
546. De Mare A, Maudsley S, Azmi A, Hendrickx JO, Opdebeeck B, Neven E, D'Haese PC, Verhulst A. Sclerostin as regulatory molecule in vascular media calcification and the bone-vascular axis. *Toxins.* 2019;11(7).
547. Shalash MAM, Rohoma KH, Kandil NS, Abdel Mohsen MA, Taha AAF. Serum sclerostin level and its relation to subclinical atherosclerosis in subjects with type 2 diabetes. *J Diabetes Complications.* 2019;33(8):592-597.
548. Ong AM, Kang K, Weiler HA, Morin SN. Fermented milk products and bone health in postmenopausal women: a systematic review of randomized controlled trials, prospective cohorts, and case-control studies. *Adv Nutr.* 2020;11(2):251-265.

549. Crichton GE & Alkerwi A. Dairy food intake is positively associated with cardiovascular health: findings from Observation of Cardiovascular Risk Factors in Luxembourg study. *Nutr Res.* 2014;34(12):1036-1044.
550. Kalem MN, Kalem Z, Akgun N, Bakırarar B. The relationship between postmenopausal women's sclerostin levels and their bone density, age, body mass index, hormonal status, and smoking and consumption of coffee and dairy products. *Arch Gynecol Obstet.* 2017;295(3):785-793.
551. Elam ML, Johnson SA, Hooshmand S, Feresin RG, Payton ME, Gu J, Arjmandi BH. A calcium-collagen chelate dietary supplement attenuates bone loss in postmenopausal women with osteopenia: a randomized controlled trial. *J Med Food.* 2015;18(3):324-331.
552. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol.* 1985;122(1):51-65.
553. Šatalić Z, Colić Barić I, Cecić I, Keser I. Short food frequency questionnaire can discriminate inadequate and adequate calcium intake in Croatian postmenopausal women. *Nutr Res.* 2007;27(9):542-547.
554. Berger C, Langsetmo L, Joseph L, Hanley DA, Davison KS, Josse R, Kreiger N, Tenenhouse A, Goltzman D. Change in bone mineral density as a function of age in women and men and association with the use of antiresorptive agents. *CMAJ.* 2008;178(13):1660-1668.
555. Osteoporosis Canada. Diagnosis. Internet: https://osteoporosis.ca/about-the-disease/diagnosis/. Accessed January 19, 2020.
556. Spurlock ME & Gabler NK. The development of porcine models of obesity and the metabolic syndrome. *J Nutr.* 2008;138(2):397-402.
557. Neeb ZP, Edwards JM, Alloosh M, Long X, Mokelke EA, Sturek M. Metabolic syndrome and coronary artery disease in Ossabaw compared with Yucatan swine. *Comp Med.* 2010;60(4):300-315.
558. Kreutz RP, Alloosh M, Mansour K, Neeb Z, Kreutz Y, Flockhart DA, Sturek M. Morbid obesity and metabolic syndrome in Ossabaw miniature swine are associated with increased platelet reactivity. *Diabetes Metab Syndr Obes.* 2011;4:99-105.
559. Abargouei AS, Janghorbani M, Salehi-Marzijarani M, Esmaillzadeh A. Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. *Int J Obes.* 2012;36(12):1485-1493.
560. Geng T, Qi L, Huang T. Effects of dairy products consumption on body weight and body composition among adults: an updated meta-analysis of 37 randomized control trials. *Mol Nutr Food Res.* 2018;62(1).
561. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c869.
562. Juszczak E, Altman DG, Hopewell S, Schulz K. Reporting of multi-arm parallel-group randomized trials: extension of the CONSORT 2010 Statement. *JAMA.* 2019;321(16):1610-1620.
563. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, Thuren T. Effects of interleukin-1beta inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. *Circulation.* 2012;126(23):2739-2748.

564. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol.* 2013;61(4):404-410.
565. Libby P. Interleukin-1 beta as a target for atherosclerosis therapy: biological basis of CANTOS and beyond. *J Am Coll Cardiol.* 2017;70(18):2278-2289.
566. Trepanowski JF & Ioannidis JPA. Perspective: Limiting dependence on nonrandomized studies and improving randomized rrials in human nutrition research: why and how. *Adv Nutr.* 2018;9(4):367-377.
567. Forman DE, Maurer MS, Boyd C, Brindis R, Salive ME, Horne FM, Bell SP, Fulmer T, Reuben DB, Zieman S et al. Multimorbidity in older adults with cardiovascular disease. *J Am Coll Cardiol.* 2018;71(19):2149-2161.
568. Nguyen TV, Rivadeneira F, Civitelli R. New guidelines for data reporting and statistical analysis: helping authors with transparency and rigor in research. *J Bone Miner Res.* 2019;34(11):1981-1984.
569. VanWormer JJ & Boucher JL. Motivational interviewing and diet modification: a review of the evidence. *Diabetes Educ.* 2004;30(3):404-419.
570. Hernandez ER, Revilla M, Seco-Durban C, Villa LF, Cortes J, Rico H. Heterogeneity of trabecular and cortical postmenopausal bone loss: a longitudinal study with pQCT. *Bone.* 1997;20(3):283-287.
571. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res.* 2017;120(7):1183-1196.
572. Zapata HJ & Quagliarello VJ. The microbiota and microbiome in aging: potential implications in health and age-related diseases. *J Am Geriatr Soc.* 2015;63(4):776-781.
573. Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, Verschoor CP, Loukov D, Schenck LP, Jury J, Foley KP et al. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host & Microbe.* 2018;23(4):570.
574. Nagpal R, Kumar M, Yadav AK, Hemalatha R, Yadav H, Marotta F, Yamashiro Y. Gut microbiota in health and disease: an overview focused on metabolic inflammation. *Benef Microbes.* 2016;7(2):181-194.
575. Barr SI. Is the 2019 Canada's Food Guide Snapshot nutritionally adequate? *Appl Physiol Nutr Metab.* 2019;44(12):1387-1390.

Appendix 1. Feedback Survey: Participation in Pilot Calcium Study – Questionnaire

Feedback Survey
Participation in Pilot Calcium Study

Please check the appropriate box:

- ❒ I was part of the Calcium Supplement Group
- ❒ I was part of the Dietary Calcium Group
- ❒ I do not know which group I was part of

Thank you for your participation in our pilot study. In part due to the data collected as a result of your one-year commitment, we received funding from the *Canadian Institutes of Health Research* to continue the study on a much larger scale. We would like to invite you to take the following survey to help us better understand how we could further improve the study for the future participants. The survey will take 15-20 minutes to complete.

1. Taking supplements and following a specific diet plan every day can be an inconvenience for some people.
    a. Was it difficult for you to take your supplement(s) every day?
        ❒ No
        ❒ Yes

        If you answered 'yes', please explain the difficulties you have encountered and what we could do to improve in the future.

        _____
        _____
        _____

2. Was it difficult for you to have the right amount dairy or calcium in your diet every day?
    ❒ No
    ❒ Yes

    If you answered 'yes', please explain the difficulties you have encountered and what we could do to improve in the future.

    _____
    _____
    _____

3. Did you feel well equipped to make the food choices necessary to have the right amount of dairy or calcium in your diet throughout the study?
    ❒ No
    ❒ Yes
    If you answered 'no', please explain why you did not feel well equipped to make the food choices necessary to have the right amount of dairy or calcium in your diet throughout the study.

    _____
    _____
    _____

4. You were contacted on a monthly basis for telephone interviews during the study. When you were asked to describe the foods that you had eaten the previous day, did you find it difficult to estimate the portion size of any foods?
    ❒ No
    ❒ Yes

If you answered 'yes', please list a few foods for which the portion sizes were difficult for you to estimate.

_____
_____
_____

5. Feedback was provided to you during your monthly telephone interviews. Overall, did you find the feedback regarding the amount of dairy or calcium in your diet useful?
   - ❒ No
   - ❒ Yes

   If you answered 'no', please explain.

   _____
   _____
   _____

6. During the study, did you consult any of the supporting documents provided to you?
   - ❒ No
   - ❒ Yes

   If you answered 'yes', please check the documents you consulted at least once.

   - ❒ Dairy Farmer's calcium calculator pamphlet
   - ❒ Sample menus
   - ❒ Nutrition label reading tool
   - ❒ Newsletter

   If you answered 'no', please explain why you did not consult the supporting documents.

   _____
   _____
   _____

7. In your opinion, what other supporting materials or communication tools would be useful for future participants in the study?
   a. Website
   b. Blogs
   c. Social media
   d. I don't know what communication tools would be useful for future participants
   e. Other: _____

8. If a newsletter would have been sent to you every three months (with updates on the study, dietary tips and recipes), would you have been interested in reading it?
   - ❒ No
   - ❒ Yes

If applicable, please briefly describe what information you would have liked to be included in the newsletter.

_____
_____
_____

9. If there was an application that you could have downloaded to your phone to help you keep track of the foods you ate, would you have used it?
   - ❒ No
   - ❒ Yes

10. Would you have liked to receive a text message or e-mail daily to remind you to take your supplement(s)?
   - ❒ No
   - ❒ Yes

11. Now that the study is over, we would like to know how your current supplement use and diet compare to before you started the study.

    a. Before the study, were you taking:
       i. Vitamin D supplements? ❒ No ❒ Yes (specify dosage): _____
       ii. Calcium supplements? ❒ No ❒ Yes (specify dosage): _____

    b. Are you currently taking:
       i. Vitamin D supplements? ❒ No ❒ Yes (specify dosage): _____
       ii. Calcium supplements? ❒ No ❒ Yes (specify dosage): _____

    c. Compared to before the study, the amount of dairy or calcium in your diet has:
       - ❒ Increased
       - ❒ Stayed the same
       - ❒ Decreased

    If either the amount of dairy or calcium in your diet, or your use of supplements has changed, please explain what made you change your habits.

    _____
    _____
    _____

12. Overall, was your participation a positive experience?
   - ❒ No
   - ❒ Yes

13. What did you like most about the study?

_____
_____
_____
_____

14. What did you like least about the study?

_____
_____
_____
_____

15. Would you recommend a friend or family member to participate in the study?
    - ❒ No
    - ❒ Yes

16. Your comments are key to help us improve and to help our future participants in the study. Please let us know if there are things that we could do to improve the experience of our future participants.

_____
_____
_____
_____
_____

Appendix 2. Feedback Survey: Participation in Pilot Calcium Study – Results (raw data)

Supplement Group responses (n=4)	Dietary Group responses (n=4)	Total responses (n=8)
Question 1: Taking supplements and following a specific diet plan every day can be an inconvenience for some people. Was it difficult for you to take your supplement(s) every day?		
100% answered No	100% answered No	100% answered No
Question 2a: Was it difficult for you to have the right amount of dairy or calcium in your diet every day?		
100% answered Yes	75% (3) answered No 25% (1) answered Yes	37.5% (3) answered No 62.5% (5) answered Yes
Question 2b: Please explain the difficulties you have encountered and what we could do to improve in the future.		
(Suppl-2): Offer tasty substitutes e.g. I craved ice cream &, after a while, I was given a coconut milk ice cream substitute that was wonderful. It was food e.g. Pizza that I craved as well. No pizza tasted the way I liked without cheese. **(Suppl-4):** Lactose intolerant so could not have much dairy and other sources not always obtainable. In the beginning I had concerns that the supplements would not provide a sufficient amount. **(Suppl-7):** It was annoying to have to measure everything. Some food that I thought was too small--for example, a tiny container of salmon--I was later told was sufficient. Sometimes, I took too much cheddar cheese.(For the below question, I would say "somewhat". It's difficult to answer some of these questions, Yes or No. **(Suppl-9):** I love my dairy & was/am used to enjoying my cafe au lait every morning as well as yogurt, cheese /in lasagna, pasta, Mexican food **(Diet-8):** I already ate a lot of dairy products and on a few occasions was borderline high on my intake of calcium although I thought I did not take enough and was trying to add more		
Question 3a: Did you feel well equipped to make the food choices necessary to have the right amount of dairy or calcium in your diet throughout the study?		
75% (3) answered Yes 25% (1) answered No* participant answered "Somewhat"	100% answered Yes	12.5% (1) answered No 87.5% (7) answered Yes
Question 3b: Please explain why you did not feel well equipped to make the food choices necessary to have the right amount of dairy or calcium in your diet throughout the study.		
(Suppl-7): See the response to the one above. Again for the below questions, I would say, somewhat. Not totally "No." However, there was no other choice. You should give more flexibility to future participants for their responses.		
Question 4a: You were contacted on a monthly basis for telephone interviews during the study. When you were asked to describe the foods that you had eaten the previous day, did you find it difficult to estimate the portion size of any foods?		
100% answered Yes	50% (2) answered Yes 50% (2) answered No	75% (6) answered Yes 25% (2) answered No
Question 4b: Please list a few foods for which the portion sizes were difficult for you to estimate.		
(Suppl-2): Salad, soup ingredients for individual portions, restaurant sandwiches.		

(Suppl-9): meat/canned tuna salad,/egg salad, green or mixed salad, rice/potatoes,dairy **(Suppl-7)**: Cheeses, principally. Also some vegetables. Again, it was annoying to have to measure the amount of meat or fish and other foods. **(Diet-3):** berries, yogurt when dispensed from large container, cheese when cut from large block or grated, vegetable **(Diet-8):** Some vegetables like cucumber where it is easy to eat several slices without any notion of the portion size. Also when cooking meals I always cooked for 2 people so I had to think about separating the amount of ingredients		
Question 5: Feedback was provided to you during your monthly telephone interviews. Overall, did you find the feedback regarding the amount of dairy or calcium in your diet useful?		
100% answered Yes	25% (1) answered No 75% (3) answered Yes	87.5% (7) answered Yes 12.5% (1) answered No
Question 6a: During the study, did you consult any of the supporting documents that were provided to you?		
100% answered Yes	100% Answered Yes	100% answered Yes
Question 6b: If you answered 'yes', please check the documents you consulted at least once.		
Dairy Farmer's Calcium Calculator		
100% answered Yes	25% (1) answered No 75% (3) answered Yes	87.5% (7) answered Yes 12.5% (1) answered No
Sample Menus		
25% (1) answered Yes 75% (3) answered No	25% (1) answered Yes 75% (3) answered No	25% (2) answered Yes 75% (6) answered No
Nutrition Label Reading Tool		
50% (2) answered Yes 50% (2) answered No	25% (1) answered No 75% (3) answered Yes	62.5% (5) answered Yes 37.5% (3) answered No
Newsletter		
25% (1) answered Yes 75% (3) answered No	25% (1) answered Yes 75% (3) answered No	25% (2) answered Yes 75% (6) answered No
Question 7: In your opinion, what other supporting materials or communication tools would be useful for future participants in the study?		
Website		
50% (2) answered Yes 50% (2) answered No	25% (1) answered No 75% (3) answered Yes	62.5% (5) answered Yes 37.5% (3) answered No
Blogs		
25% (1) answered Yes 75% (3) answered No	100% answered No	12.5% (1)answered Yes 87.5% (7) answered No

Social Media		
25% (1) answered Yes 75% (3) answered No	100% answered No	12.5% (1) answered Yes 87.5% (7) answered No
I don't know what communication tools would be useful for future participants		
25% (1) answered Yes 75% (3) answered No	25% (1) answered Yes 75% (3) answered No	25% (2) answered Yes 75% (6) answered No
Other (open)		
"Sharing tips with others in the study" "E-mail and telephone. Not everyone uses social media."		
Question 8a: If a newsletter would have been sent to you every three months (with updates on the study, dietary tips and recipes), would you have been interested in reading it?		
75% (3) answered Yes 25% (1) answered No	100% answered Yes	12.5% (1) answered No 87.5% (7) answered Yes
Question 8b: Please briefly describe what information you would have liked to be included in the newsletter.		
(Suppl-2): Questions from participants with answers from the related professionals. **(Suppl-4):** Conclusions to date - similar studies **(Suppl-9):** Photos of real-sized plates of food with sample meals showing real-sized portions. **(Diet-6):** I would have liked actual info on the study - number of participants in each group, were any trends noticed. Info on the tests being done such as why were these particular tests done , what was being looked for, etc, Recipes are always good. **(Diet-3):** the above info would be good **(Diet-5):** All the benefits of eating healthy **(Diet-8)**: How the recruitement for the study went, any data obtained from an interim analysis, difficulties by other participants and how to overcome them, tips and success stories		
Question 9: If there was an application that you could have downloaded to your phone to help you keep track of the foods you ate, would you have used it?		
50% (2) answered Yes 50% (2) answered No	25% (1) answered Yes 75% (3) answered No	37.5% (3) answered Yes 62.5% (5) answered No
Question 10: Would you have liked to receive a text message or e-mail daily to remind you to take your supplement(s)?		
25% (1) answered Yes 75% (3) answered No	100% said No	12.5% (1) answered Yes 87.5% (7) answered No
Question 11a(i): Before the study, were you taking vitamin D supplements?		
75% (3) answered Yes 25% (1) answered No	75% (3) answered Yes 25% (1) answered No	25% (2) answered No 75% (6) answered Yes
Question 11a(i): Please specify the dosage of vitamin D you were taking.		

(Suppl-2): 10,000 x week **(Suppl-4)**: 400 IU **(Suppl-9):** 400 units daily **(Suppl-7):** None	**(Diet-6):** 1000 IU - 1800 IU **(Diet-3)**: None **(Diet-5):** 1 a day 400 IU **(Diet-8):** 1000 mg per day	
Question 11a(ii): Before the study, were you taking calcium supplements?		
75% (3) answered Yes 25% (1) answered No	50% (2) answered Yes 50% (2) answered No	62.5% (5) answered Yes 37.5% (3) answered No
Question 11a(ii): Please specify the dosage of calcium supplement you were taking.		
(Suppl-2): 2 x 500 daily **(Suppl-4)**: 333 mg **(Suppl-9):** calcium, mg can't remember **(Suppl-7):** None	**(Diet-6):** 500 twice a day **(Diet-3):** None **(Diet-5)**: 2 per day **(Diet-8):** None	
Question 11b(i): Are you currently taking vitamin D supplements?		
75% (3) answered Yes 25% (1) answered No	100% answered Yes	12.5% (1) answered No 87.5% (7) answered Yes
Question 11b(i): Please specify the dosage of vitamin D you are currently taking.		
(Suppl-2): 400 IU daily **(Suppl-4)**: 400 IU **(Suppl-9):** 800 units **(Suppl-7):** None	**(Diet-6):** 1000 IU every other day **(Diet-3):** 400 IU **(Diet-5):** 1 a day 400 IU **(Diet-8):** 1000 mg per day	
Question 11b(ii): Are you currently taking calcium supplements?		
75% (3) answered Yes 25% (1) answered No	100% answered No	62.5% (5) answered No 37.5% (3) answered Yes
Question 11b(ii): Please specify the dosage of calcium you are currently taking.		
(Suppl-2): 500 1 x day **(Suppl-4):** 333 mg **(Suppl-9):** 500 units **(Suppl-7):** None	**(Diet-6)**: None **(Diet-3):** None **(Diet-5):** None **(Diet-8):** None	
Question 11c: Compared to before the study, the amount of dairy or calcium in your diet has:		
25% (1) answered Decreased 25% (1) answered Increased 50% (2) answered Stayed the Same	25% (1) answered Increased 75% (3) answered Stayed the Same	12.5% (1) answered Decreased 25% (2) answered Increased 62.5% (5) answered Stayed the Same
Question 11c: If either the amount of dairy or calcium in your diet, or use of supplements has changed, please explain what made you change your habits.		

(Suppl-2): Decreased. Fat content in dairy products & realization that more calcium may have a negative effect on my health. **(Suppl-4):** Stayed the same. **(Suppl-9):** Increased. Found a bottle of calcium tablets from study. Taking 1 a day. I just love dairy but am not consistent in its use. Some days only tea with a little milk, no yogurt or cheese, other days a lot of the 3 mentioned. A matter of time, taste, what's in the fridge or cupboard...no particular reason. Some days I forget to take the supplement. **(Suppl-7)**:Stayed the same.	**(Diet-6):** Increased. My bad cholesterol dropped by 10% by drinking 3 glasses of soy milk daily. **(Diet-3)**: Stayed the same. I decided to use Vit.D over the winter months for now as I do spend time outside in warmer months. **(Diet-5)**: Stayed the same. I have learned to eat a balance of food for my Calcium **(Diet-8):** Stayed the same.	
Question 12: Overall, was your participation a positive experience?		
100% answered Yes	100% answered Yes	100% answered Yes
Question 13: What did you like most about the study?		
(Suppl-2): Commitment to reading food ingredient %. Ability to have arterial ultrasounds when it wouldn't be available during my annual general checkup. Above all, happy to donate my body for scientific research that will ultimately benefit all. **(Suppl-4):** Keeping track of what I ate. **(Suppl-9):** Patience & concern of the people running the study. Free supplements as I'm on a low fixed income, the thorough before & after testing & the opportunity to get out & about. **(Suppl-7)**: Becoming more conscious of sources of calcium. **(Diet-6):** I liked the idea of contributing to women's health knowledge. **(Diet-3):** I think that studies are important in improving and hopefully bettering our health/lifestyle practices. **(Diet-5):** I learned to eat a balance of calcium foods, but I always ate very healthy **(Diet-8)**: Made me more conscious of how much calcium I was taking in my diet		
Question 14: What did you like least about the study?		
(Suppl-2): Restrictions on favorite foods high in calcium. **(Suppl-4):** No individual feedback (i.e., was not made aware of how my results compared to the "norm" assuming that there was one) **(Suppl-9):** The early morning visits. **(Suppl-7):** Having to measure food. **(Diet-6)**: Having to deal with the dietitians - they seemed to think that having the weight or size of ingredients that went into the dish & then the weight of portion that I ate was not sufficient info for them. **(Diet-3):** Having to remember to take my supplements and take the appropriate amt. of dietary calcium daily. It was challenging for me as any change in habits can be. **(Diet-5):** I liked all the study , there was nothing I didn't like **(Diet-8):** at the beginning I was not getting any feedback on how much calcium was in my diet and if I was on target, too low or too high		
Question 15: Would you recommend a friend or a family member to participate in the study?		
25% (1) answered No	100% answered Yes	12.5% (1) answered No

75% (3) answered Yes		87.5% (7) answered Yes
Question 16: General Comments		
(Suppl-2): Hand out research proposal & related articles. Offer a buddy system or participant social network for support & idea sharing. **(Suppl-9):** I think you & your team did a magnificent job. However, the feedback questions should have had a 3rd choice: possibly or maybe or not sure, would have been more accurate in my opinion. Several of the questions needed that. I'm not a "black or white' type so maybe it's just me. Timing was not the best either. This is a particularly busy, stressful season & one more thing to accomplish can be overwhelming. After the holiday would have been more helpful for me. Thanks for all the care, concern & patience you all demonstrated. **(Suppl-7):** Please pay them more money. **(Diet-6):** I think that the exact nature of what the dietitians are looking for should be explained up front. **(Diet-3):** Smart phone alerts or reminders might be helpful for those who are more techy. Scheduling visit dates or confirming visit calls ahead so that you can note or recall your previous food intake better. **(Diet-5):** I can't comment, I found the study very helpful **(Diet-8):** There were a lot of measurements taken during the study, as a participant I would like to know what are the conclusion of the study on these other parameters and do I need to change something in my diet to stay or become healthier.		

Appendix 3. Educational resources developed for participants of the Calcium Study

Participant Information Sheet

You have been selected by chance to be in the **Dietary Calcium Group.**
For the duration of the 1-year study, we ask that you:

1. Take 1 capsule of vitamin D supplement (400 IU) after your first meal of the day.

2. Increase the amount of foods rich in calcium in your diet to 3 servings per day.

What foods are rich in calcium?

For the purpose of this study, foods that contain 250-300 mg of calcium per serving, or 25-30% of your % daily value of calcium per serving, are considered rich in calcium. These include:

A. Dairy

All foods that are made from milk are considered rich in calcium, but some contain more calcium than others. Here is a list of dairy foods and examples of what is considered 1 serving of each:

Food		Serving size
Milk	...	1 cup or 250 ml
Plain yogurt	...	¾ cup or 175 ml
Fruit flavoured yogurt	...	1 cup or 250 ml
Soft cheese *(blue, feta)*	...	50g or the size of 3 stacked dice
Firm cheese *(cheddar, Swiss, Gouda)*	...	35g or the size of 2 stacked dice
Parmesan cheese	...	4 Tbsp or 60 ml grated

B. Non-dairy foods

Some foods that are not made from milk still contain calcium either naturally or because they are fortified. Again, certain foods contain more calcium than others. Here is a list of non-dairy foods and examples of what is considered **½ serving** of each:

Food		Serving size
Tofu *(made with calcium)*	...	100 g or 100 ml
Salmon, canned, with bones	...	70 g or ⅓ of a can
Alternative milk beverage fortified with calcium *(soy, almond, rice, orange juice)*	...	½ cup or 125 ml

Please note that the portion sizes presented are estimates. It is important to always refer to the Nutrition Facts table of packaged foods that are rich in calcium for more accurate information on their calcium content.

C. Other foods rich in calcium

There are other foods that contain calcium, but the calcium content in a typical serving of these foods is much lower than 250-300 mg. Here is a list of other foods that contain calcium. If you eat **more than the amount indicated per day** for any of these foods, please let the research team know. In some cases it may be necessary to count these foods towards your servings of calcium rich foods.

Food		Amount	Food		Amount
Collard greens, cooked	…	¼ cup	Kale, bok choy, okra, cooked	…	½ cup
Baked beans	…	½ cup	Broccoli	…	1 cup
Soybeans, cooked	…	½ cup	Almonds	…	¼ cup
White beans, cooked	…	½ cup	Blackstrap molasses	…	1 tsp

What did I eat yesterday?

As a participant of the Calcium study, you will be asked "What did you eat yesterday?" at least once a month. To help you best report the foods you eat, here are some details we would like you to keep in mind.

1. How was it prepared?
2. What was the brand?
3. What was the variety?
4. How much did you eat?

Are there any tips that will help me estimate the amount of food that I ate?

- Measure the amount of foods you eat often at least once.
- Pay attention to the recipe of foods you prepare and the portion of the recipe that you ate.
- Read the packaging, such as the container or wrapper, of packaged foods.
- Compare the amount of food you ate to the size of common household items.

When should I contact the research team?

Please advise a member of the research team if you become ill or before you start to take any new medications, vitamins, or supplements.

Study Co-ordinator: Michelle Wall……... (514) 934-1934 ext. 45742
Study Dietitian: Angel Ong……... (514) 934-1934 ext. 43715

Your 1-week phone call will take place on: _____

Your 6-month follow-up visit will be scheduled in: _____

Participant Information Sheet

You have been selected by chance to be in the **Supplemental Calcium Group.**

For the duration of the 1-year study, we ask that you:

3. Take 2 tablets of calcium supplement (250 mg each) and 1 capsule of vitamin D supplement (800 IU) after your first meal of the day.
4. Take 1 tablet of calcium supplement (250 mg) in the evening.
5. Limit the amount of foods rich in calcium in your diet to 1 serving per day.

What foods are rich in calcium?

For the purpose of this study, foods that contain 150-200 mg of calcium per serving, or 15-20% of your % daily value of calcium per serving, are considered rich in calcium. These include:

D. Dairy

All foods that are made from milk are considered rich in calcium, but some contain more calcium than others. Here is a list of dairy foods and examples of what is considered **1 serving** of each:

Food		Serving size	Food		Serving size
Milk	...	⅔ cup or 160 ml	Soft cheese *(blue, feta)*	...	30 g or size of a thumb
Plain yogurt	...	½ cup or 125 ml			
Fruit flavoured yogurt	...	¾ cup or 175 ml	Firm cheese *(cheddar, Swiss, Gouda)*	...	25 g or 3 tbsp diced

E. Non-dairy foods

Some foods that are not made from milk still contain calcium either naturally or because they are fortified. Again, certain foods contain more calcium than others. Here is a list of non-dairy foods and examples of what is considered **1 serving** of each:

Food		Serving size
Tofu *(made with calcium)*	...	100 g or 100 ml
Salmon, canned, with bones	...	70 g or ⅓ of a can
Alternative milk beverage fortified with calcium *(soy, almond, rice, orange juice)*	...	⅔ cup or 160 ml

Please note that these serving sizes are estimates. It is important to always refer to the Nutrition Facts table of packaged foods that are rich in calcium for more accurate information on their calcium content.

F. Other foods that contain calcium

There are other foods that contain calcium, but the calcium content in a typical serving of these foods is much lower than 150-200 mg. Here is a list of other foods that contain calcium. If you eat **more than the amount indicated per day** for any of these foods, please let the research team know. In some cases it may be necessary to count these foods towards your servings of calcium rich foods.

Food		Amount	Food		Amount
Collard greens, cooked	…	¼ cup	Kale, bok choy, okra, cooked	…	½ cup
Baked beans	…	½ cup	Broccoli	…	1 cup
Soybeans, cooked	…	½ cup	Almonds	…	¼ cup
White beans, cooked	…	½ cup	Blackstrap molasses	…	1 tsp

What did I eat yesterday?

As a participant of the Calcium study, you will be asked "What did you eat yesterday?" at least once a month. To help you best report the foods you eat, here are some details we would like you to keep in mind.

5. How was it prepared?
6. What was the brand?
7. What was the variety?
8. How much did you eat?

Are there any tips that will help me estimate the amount of food I ate?

- Measure the amount of foods you eat often at least once.
- Pay attention to the recipe of foods you prepare and the portion of the recipe that you ate.
- Read the packaging, such as the container or wrapper, of packaged foods.
- Compare the amount of food you ate to the size of common household items.

When should I contact the research team?

Please advise a member of the research team if you become ill or before you start to take any new medications, vitamins, or supplements.

Study Co-ordinator: Michelle Wall…….... (514) 934-1934 ext. 45742
Study Dietitian: Angel Ong…….... (514) 934-1934 ext. 43715

Your 1-week phone call will take place on: _____

Your 6-month follow-up visit will be scheduled in: _____

Participant Information Sheet

You have been selected by chance to be in the **Usual Diet Group.**

For the duration of the 1-year study, we ask that you:

1. Take 1 capsule of vitamin D supplement (400 IU) every morning after your first meal of the day.

2. Continue with your usual diet.

What did I eat yesterday?

As a participant of the Calcium study, you will be asked "What did you eat yesterday?" at least once a month. To help you best report the foods you eat, here are some details we would like you to keep in mind.

1. How was it prepared?
2. What was the brand?
3. What was the variety?
4. How much did you eat?

Are there any tips that will help me estimate the amount of food I ate?

- Measure the amount of foods you eat often at least once.
- Pay attention to the recipe of foods you prepare and the portion of the recipe that you ate.
- Read the packaging, such as the container or wrapper, of packaged foods.
- Compare the amount of food you ate to the size of common household items.

When should I contact the research team?

Please advise a member of the research team if you become ill or before you start to take any new medications, vitamins, or supplements.

Study Co-ordinator: Michelle Wall……... (514) 934-1934 ext. 45742
Study Dietitian: Angel Ong……... (514) 934-1934 ext. 43715

Your 1-week phone call will take place on: _____
Your 6-month follow-up visit will be scheduled in: _____

I should be looking at the Nutrition Facts table for what specific food?

It is good to take a look at the Nutrition Facts table of foods you eat to learn whether that food is rich in calcium or not.

You can also compare the labels of similar products to see which variety can help you meet your calcium target more easily.

Some foods that vary in calcium content depending on the type and brand. Examples include:

- Cheeses
- Yogurts
- Pre-packaged foods that contain dairy products like cheese or milk

Should I aim to reach 100% DV for calcium from foods rich in calcium?

No. You get calcium in your diet from a variety of foods. Most of the foods that you eat, other than the ones that are high in calcium, also have some calcium in them, and we have taken that amount into account. So, instead of aiming for 100% DV of calcium, you should focus on your target of 3 servings of calcium rich foods a day.

If you have questions or would like to have more information on how to read the Nutrition Facts table, please contact:

Angel Ong, the study dietitian
514-934-1934 extension 43715
angel.ong@mail.mcgill.ca.

Institut de recherche Centre universitaire de santé McGill
Research Institute McGill University Health Centre

CIHR IRSC

March 6 2014 V.1

CALCIUM

A STUDY ON THE IMPACT OF CALCIUM ON WOMEN'S VASCULAR HEALTH

Principal co-investigators:
Dr. Suzanne Morin, MD MSc
Dr. Stella Daskalopoulou, MD PhD

The Nutrition Facts Table

A useful tool to help you meet your Calcium target

Prepared for participants in the Dietary Calcium Group

What is my dietary calcium target?

Participants, such as yourself, who have been assigned to the dietary calcium group of the study, have a dietary calcium target of three (3) servings of foods rich in calcium per day.

What foods are rich in calcium?

Many foods contain calcium, but some contain more than others. Some foods rich in calcium include:

- All dairy foods (foods made from milk)
- Non-dairy foods such as beverages fortified with calcium (soy, orange juice), some tofu, salmon or sardines canned with bones.
- Other foods like some leafy greens, legumes, and almonds.

How much calcium is in one serving of foods rich in calcium?

For the purpose of this study, one serving is equal to approximately 300 mg of calcium. You can find specific examples in another document called the *Participant Information Sheet*.

Do I need to calculate the amount of calcium I eat?

No. You are not expected to count the amount of calcium in your diet, but it is important for you to know whether the food that you eat contains enough, too little, or too much calcium with respect to your daily target. You can read the Nutrition Facts table to learn more about the calcium content of some of the foods rich in calcium that you eat.

What is a Nutrition Facts table?

The Nutrition Facts table appears on the label of almost all pre-packaged foods. It gives you information on calories and nutrients, including calcium.

What should I be looking for when I read the Nutrition Facts table?

Step 1. Look at the serving size

Nutrition Facts are based on a specific amount of food.

Step 2. Read the % DV for Calcium

The % DV helps you see if the indicated serving size has a little or a lot of calcium. It will also help you determine how many servings of foods rich in calcium you are having if you consume that specific amount.

Step 3. Compare the % DV to your target

If you eat more or less than the amount shown in the Nutrition Facts table, then the amount of Calcium you have will be different than what is shown in the table. Compare this to the amount you actually eat to reach your daily target.

1 serving = 25-30% DV

You need 3 servings to reach your daily target.

Here's an example of a Nutrition Facts table:

Valeur nutritive Nutrition Facts	
pour 3/4 tasse (175g) Per 3/4 tasse (175g)	
Teneur **Amount**	**% valeur quotidienne** **% Daily Value**
Calories / Calories 90	
Lipides / Fat 3.5 g	5 %
saturés / Saturated 2 + trans / Trans 0	11%
Cholestérol / Cholesterol 10 mg	
Sodium / Sodium 85 mg	4 %
Glucides / Carbohydrates 5 g	2 %
Fibres / Fibre 0	0 %
Sucres / Sugars 5 g	
Protéines / Protein 10 g	
Vitamine A / Vitamin A	4 %
Vitamine C / Vitamin C	2 %
Calcium / Calcium	25%
Fer / Iron	0 %

I should be looking at the Nutrition Facts table for what specific food?

It is good to take a look at the Nutrition Facts table of foods you eat to learn whether that food is rich in calcium or not.

You can also compare the labels of similar products to see which variety can help you meet your calcium target more easily.

Some foods vary in calcium content depending on the type and brand. Examples include:

- Cheeses
- Yogurts
- Pre-packaged foods that contain dairy products like cheese or milk

Is it possible that I don't have enough calcium?

You are getting 750 mg of calcium from supplements and approximately 450 mg in your diet from a variety of foods. Most of the foods that you eat, other than the ones that are rich in calcium, also have some calcium in them, and we have taken that amount into account. By reaching your daily target of 1 serving of foods rich in calcium while taking the calcium supplements, you will be meeting the recommended needs of 1200 mg per day.

If you have questions or would like to have more information on how to read the Nutrition Facts table, please contact:

Angel Ong, the study dietitian
514-934-1934 extension 43715
angel.ong@mail.mcgill.ca.

Institut de recherche Centre universitaire de santé McGill
Research Institute McGill University Health Centre
CIHR IRSC

March 6 2014 V.1

CALCIUM

A STUDY ON THE IMPACT OF CALCIUM ON WOMEN'S VASCULAR HEALTH

Principal co-investigators:
Dr. Suzanne Morin, MD MSc
Dr. Stella Daskalopoulou, MD PhD

The Nutrition Facts Table

A useful tool to help you meet your Calcium target

Prepared for participants in the Calcium Supplement Group

What is my dietary calcium target?

Participants, such as yourself, who have been assigned to the calcium supplement group of the study, have a dietary calcium target of one (1) serving of foods rich in calcium per day.

What foods are rich in calcium?

Many foods contain calcium, but some contain more than others. Some foods rich in calcium include:

- All dairy foods (foods made from milk)
- Non-dairy foods such as beverages fortified with calcium (soy, orange juice), some tofu, salmon or sardines canned with bones.
- Other foods like some leafy greens, legumes, and almonds.

How much calcium is in one serving of foods rich in calcium?

For the purpose of this study, one serving is equal to approximately 150-200 mg of calcium. You can find specific examples in another document called the *Participant Information Sheet*.

Do I need to calculate the amount of calcium in my diet?

You are not expected to count the amount of calcium in your diet, but it is important for you to know whether the food that you eat contains enough, too little, or too much calcium with respect to your daily target. You can read the Nutrition Facts table to learn more about the calcium content of some of the foods rich in calcium that you eat.

What is a Nutrition Facts table?

The Nutrition Facts table appears on the label of almost all pre-packaged foods. It gives you information on calories and nutrients, including calcium.

What should I be looking for when I read the Nutrition Facts table?

Step 1. Look at the serving size

Nutrition Facts are based on a specific amount of food.

Step 2. Read the % DV for Calcium

The % DV helps you see if the indicated serving size has a little or a lot of calcium. It will also help you determine how many servings of foods rich in calcium you are having if you consume that specific amount.

Step 3. Compare the % DV to your target

If you eat more or less than the amount shown in the Nutrition Facts table, then the amount of Calcium you have will be different than what is shown in the table. Compare this to the amount you actually eat to reach your daily target.

Daily target:

1 serving = 15-20% DV

You need 1 serving to reach your daily target.

Here's an example of a Nutrition Facts table:

Valeur nutritive Nutrition Facts	
pour 3/4 tasse (175g) Per 3/4 tasse (175g)	
Teneur **Amount**	**% valeur quotidienne** **% Daily Value**
Calories / Calories 170	
Lipides / Fat 3g	5 %
saturés / Saturated 2g + trans / Trans 11	0g%
Cholestérol / Cholesterol 10mg	
Sodium / Sodium 55mg	2 %
Glucides / Carbohydrates 22g	7 %
Fibres / Fibre 1g	4 %
Sucres / Sugars 20g	
Protéines / Protein 13g	
Vitamine A / Vitamin A	2 %
Vitamine C / Vitamin C	15%
Calcium / Calcium	15%
Fer / Iron	2 %