Association of Dietary Calcium Intake and Cardiovascular Markers in Healthy Postmenopausal Women

Shubhabrata Das

Faculty of Medicine, Division of Experimental Medicine
McGill University, Montreal

Submitted: August 2018

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master Science

TABLE OF CONTENTS

ACKNOWLEDGEMENTS 4

ABSTRACT

English 5

French 7

ABBREVIATIONS 9

1.0 BACKGROUND 11

1.1 Cardiovascular Disease 11

1.2 Cardiovascular Disease and Menopause 11

1.2.1 Mechanisms of Increased Cardiovascular risk in Postmenopausal Women 12

1.3 Menopause, Osteoporosis and Calcium Metabolism 13

1.3.1 Postmenopausal Osteoporosis 13

1.3.2 Calcium Metabolism 14

1.3.3 Calcium Intake and Osteoporosis 16

1.4 Calcium Intake and Cardiovascular Health 17

1.4.1 Supplemental Calcium and Cardiovascular Outcomes 17

1.4.2 Dietary Calcium and Cardiovascular Outcomes 19

1.4.3 Total Calcium and Cardiovascular Outcomes 20

1.4.4 Possible Harmful Mechanisms Linking Calcium Intake and Cardiovascular Health 21

1.5 Arterial Stiffness and Hemodynamics 23

1.5.1 Pathophysiology of Arterial Stiffness 23

1.5.2 Assessment of Arterial Stiffness 25

1.5.3 Calcium Intake and Arterial Stiffness Parameters 33

1.6 Carotid Intima-media Thickness 34

1.6.1 Anatomy and Histopathology 34

1.6.2 Carotid Intima-media Thickness and Cardiovascular Health 36

1.6.3 Reference and normal values of Carotid Intima-media Thickness 39

1.6.4 Calcium intake and Carotid Intima-media Thickness 39

1.7 Calcium Intake and Other Cardiovascular Markers 40

1.7.1 Calcium Intake and Serum lipids 40

1.7.2 Calcium Intake and Blood pressure 41

1.7.3 Calcium Intake and Vascular biomarkers 43

2.0 OBJECTIVES & HYPOTHESIS 46

3.0 METHODS 47

3.1 Study Design 47

3.2 Research Subjects 47
3.2.1 Recruitment and Screening 47
3.2.2 Study Population 48
3.3 Assessment Day 48
3.3.1 Assessment of Dietary Calcium 49
3.3.2 Assessment of Physical Activity 49
3.3.3 Anthropometric Measurements 49
3.3.4 Vascular Studies 49
3.3.5 Assessment of Biomarkers 52
3.4 Statistical Analyses 53
4.0 RESULTS 54
4.1 Analysis of Vascular Parameters (Main Study) 54
4.1.1 Participant Characteristics 54
4.1.2 General Characteristics of Participants across Dietary Calcium Groups 54
4.1.3 Vascular Parameters across Dietary Calcium Groups 55
4.2 Analysis of Serum Lipids (Secondary Study) 56
4.2.1 Participant Characteristics 56
4.2.2 General Characteristics of Participants across Dietary Calcium Groups 57
4.2.3 Serum Lipids across Dietary Calcium Groups 57
4.3 Main Study: Exploratory Analysis 58
4.4 Subgroup Analysis 58
TABLES 61
5.0 DISCUSSION 70
5.1 Main Study and Subgroup Analysis: Analysis of Vascular Parameters 70
5.2 Secondary Study: Analysis of Biomarkers 79
6.0 LIMITATIONS 85
7.0 CONCLUSION 86
8.0 FUTURE DIRECTIONS 87
APPENDICES 88
REFERENCES 101

ACKNOWLEDGEMENTS

I express my sincere appreciation to my supervisors, Dr. Stella S. Daskalopoulou and Dr. Suzanne N. Morin, for their constant guidance, encouragement and support for the last two and half years. Being guided by them has been a memorable learning experience that I will cherish forever.

I would like to express my gratitude towards the Vascular Health Unit laboratory manager, Yessica-Haydee Gomez, for not only helping me learn techniques of vascular studies and other experiments, but also for being there whenever needed. To the other Vascular Health Unit laboratory members especially Jessica Gorgui and Alexandra Cooke, I cannot thank them enough for helping me learn the techniques of vascular studies, including arterial stiffness assessment and carotid ultrasound, as well as for their continuing support. I also want to thank the members of Dr. Morin's laboratory, Angel M. Ong, Michelle Wall, Kristina Parsons, and Sumra Kureishy, for helping me with the study, specifically with subject recruitment, study participant interviews, data input, and the Calcium Study database.

I would like to acknowledge our administrative staff, Helena Corredor.

I also extend my thanks to my committee members, Dr. Kaberi Dasgupta and Dr. David Goltzman and, my advisor Dr. Siham Sabri, for their guidance and valuable insight on my thesis project.

I am also thankful to the Canadian Institutes of Health Research and the Dairy Farmers of Canada for the funding of our study.

Lastly, I want to thank my family without whose unconditional support I would not be who I am today.

ABSTRACT

English

Introduction: Cardiovascular disease (CVD) is one of the leading causes of death in women. Loss of ovarian function and endogenous estrogen deficiency predisposes postmenopausal women to CVD and osteoporosis. Calcium has been recommended for the prevention and treatment of osteoporosis. However, the effect of calcium intake, both from supplements as well as dietary sources, on cardiovascular (CV) health remains uncertain and largely dependent on study design and population. Carotid intima-media thickness (cIMT), arterial stiffness and hemodynamic parameters can detect CVD at a very early stage with high predictive value. Therefore, in this study, we examined the association between dietary calcium intake and CV markers, including cIMT, arterial stiffness and hemodynamics, and serum lipids, in healthy postmenopausal women.

Methods: Healthy postmenopausal women without CV risk factors and not taking calcium or vitamin D supplements were included in this study. Ninety-six postmenopausal women were included for vascular assessment (Main Study), whereas 80 participants were included for assessment of serum lipids (Secondary Study). Dietary calcium (dCa) and dietary vitamin D (dvitD) intake were evaluated by a validated food frequency questionnaire to estimate usual intake in the previous month. All participants underwent cIMT, as well as arterial stiffness measurements, including carotid to femoral pulse wave velocity (cfPWV) and other hemodynamic measurements in the early morning. Fasting blood samples were collected for assessment of serum biomarkers, including lipids. CV markers were compared across <600, 600-1000 and >1000 mg/d dCa intake.

We performed an exploratory analysis of 600-1000 mg/d dCa group as this group had the most favourable vascular markers. Furthermore, we conducted a subgroup analysis comparing CV parameters

across <800, 800-1000 and >1000 mg/d dCa intake as the group with 800-1000 mg/d dCa showed the best CV marker values in the exploratory analysis.

Results: The mean (±standard deviation) age and body mass index of our study population were 60.2±6.3 years and 25.6±3.9 kg/m^2, respectively. Although statistically non-significant, we noted favorable values of CV markers in 600-1000 mg/d dCa group compared to the extreme groups (<600 mg/d or >1000 mg/d) in the primary analysis.

Although there was no significant associations, we noted a tendency for improved CV marker values in those with <1000 mg/d vs. >1000 mg/d dCa, as well those in the middle groups of (600-1000 mg/d or 800-1000 mg/d) compared to the extreme groups (<600 mg/d, <800 mg/d or >1000 mg/d in further analyses.

Conclusion: Although the present study does not suggest significant associations between dCa and CV markers in healthy postmenopausal women, our results indicate that mid-spectrum levels of dCa intake, as compared to the lower and higher extremes, might be associated with better values of CV markers. Our ongoing randomized controlled trial will allow for a better evaluation of the effect of dCa on CV health.

ABSTRACT

French

Introduction: La maladie cardiovasculaire (MCV) est l'une des principales causes de décès chez les femmes. La perte de la fonction ovarienne et la carence en œstrogènes endogènes prédisposent les femmes ménopausées aux maladies cardiovasculaires et à l'ostéoporose. Le calcium a été recommandé pour la prévention et le traitement de l'ostéoporose. Cependant, l'effet de l'apport en calcium, provenant à la fois des suppléments et des sources alimentaires, sur la santé cardiovasculaire (CV) demeure incertain et dépend largement de la conception de l'étude et de la population. L'épaisseur de l'intima-média carotidienne (EIMc), la rigidité artérielle et les paramètres hémodynamiques, ainsi que certains lipides sériques peuvent détecter la MCV à un stade très précoce avec une valeur prédictive élevée. Par conséquent, dans cette étude, nous avons examiné l'association entre l'apport alimentaire en calcium et les marqueurs CV, y compris la EIMc, la rigidité artérielle et les paramètres hémodynamique, et les lipides sériques, chez les femmes ménopausées en bonne santé.

Méthodes: Des femmes ménopausées en bonne santé sans facteurs de risque CV et ne prenant pas de suppléments de calcium ou de vitamine D ont été incluses dans cette étude. Quatre-vingt-seize femmes ménopausées ont été incluses pour l'évaluation vasculaire (étude principale), tandis que 80 participantes ont été incluses pour l'évaluation des lipides sériques (étude secondaire). L'apport en calcium alimentaire (CaA) et en vitamine D alimentaire (vitDA) a été évalué à l'aide d'un questionnaire de fréquence alimentaire validé pour estimer l'apport habituel au cours du mois précédent. Toutes les participantes ont subi une évaluation de l'EIMc, ainsi que des mesures de rigidité artérielle, y compris la vitesse de prpopagation l'onde de pouls carotido-fémorale et d'autres mesures hémodynamiques tôt le matin. Des échantillons de sang à jeun ont été prélevés pour l'évaluation des biomarqueurs sériques, y compris les lipides. Les marqueurs CV ont été comparés entre <600, 600-1000 et >1000 mg/jour CaA.

Nous avons effectué une analyse exploratoire de 600-1000 mg / j groupe dCa car ce groupe avait les marqueurs vasculaires les plus favorables. De plus, nous avons effectué une analyse en sous-groupes comparant les paramètres CV pour des apports en dCa <800, 800-1000 et> 1000 mg / j, le groupe présentant 800-1000 mg / j dCa présentant les meilleures valeurs de marqueur CV dans l'analyse exploratoire.

Résultats: L'âge moyen (± écart-type) et l'indice de masse corporelle de notre population étudiée étaient de 60,2 ± 6,3 ans et de 25,6 ± 3,9 kg/m2, respectivement. Bien que statistiquement non significatif, nous avons noté des valeurs favorables de marqueurs CV dans 600-1000 mg / j groupe dCa par rapport aux groupes extrêmes (<600 mg / j ou> 1000 mg / j) dans l'analyse primaire. Bien qu'il n'y ait pas d'associations significatives, nous avons noté une tendance à l'amélioration des valeurs des marqueurs CV chez les sujets ayant <1000 mg / j vs> 1000 mg / j dCa, ainsi que ceux des groupes du milieu (600-1000 mg / j ou 800 -1000 mg / j) par rapport aux groupes extrêmes (<600 mg / j, <800 mg / j ou> 1000 mg / j dans d'autres analyses.

Conclusion: Bien que la présente étude ne suggère pas d'associations significatives entre le CaA et les marqueurs CV chez les femmes ménopausées en bonne santé, nos résultats indiquent que des niveaux moyens de l'apport en CaA, comparativement aux extrêmes inférieurs et supérieurs, pourraient être associés à de meilleures valeurs des marqueurs CV. Notre essai randomisé contrôlé présentement en cours permettra une meilleure évaluation de l'effet du CaA sur la santé cardiovasculaire.

ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
Apo A	Apolipoprotein A
Apo B	Apolipoprotein B
ACEI	Angiotensin converting enzyme inhibitors
AIx	Augmentation index
AIx@75	Augmentation index corrected for a heart rate of 75 beats per minute
AP	Augmentation pressure
ARB	Angiotensin receptor blocker
ARIC	Atherosclerosis Risk in Communities
ASE	American Society of Echocardiography
baPWV	Brachial ankle pulse wave velocity
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
cDBP	Central diastolic blood pressure
cPP	Central pulse pressure
cSBP	Central systolic blood pressure
cfPWV	Carotid to femoral pulse wave velocity
crPWV	Carotid to radial pulse wave velocity
cIMT	Carotid intima-media thickness
Ca^{2+}	Ionised calcium
CaMos	Canadian Multicentre Osteoporosis Study
CAIFOS	Calcium Intake Fracture Outcome study
CAPS	Carotid Atherosclerosis Progression Study
CCA	Common carotid artery
CCB	Calcium channel blockers
CHD	Coronary heart disease
CHS	Cardiovascular Health Study
CI	Confidence interval
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DBP	Diastolic blood pressure
DPTI	Diastolic pressure time index
ECG	Electrocardiogram
ERT	Estrogen replacement therapy
hs-CRP	High sensitive C-reactive protein
HDL-C	High density lipoprotein cholesterol
HR	Heart rate
HR	Hazard ratio
HRT	Hormone replacement therapy
ICAM	Intracellular cell adhesion molecule
IDL-C	Intermediate density lipoprotein cholesterol
IL	Interleukin

IPAQ	International physical activity questionnaire
JUPITER	Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin
KNHANES	Korea National Health and Nutrition Examination Survey
LDL-C	Low density lipoprotein cholesterol
MAP	Mean arterial blood pressure
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
NHANES	National Health and Nutrition Examination Survey
NIH-AARP	National Institutes of Health–American Association of Retired Persons
NHS	Nurses' Health Study
OPG	Osteoprotegerin
pPP	Peripheral pulse pressure
pDBP	Peripheral diastolic blood pressure
PP	Pulse pressure
pSBP	Peripheral systolic blood pressure
PPA	Pulse pressure amplification
PTH	Parathyroid hormone
PTT	Pulse transit time
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RAAS	Renin-Angiotensin-Aldosterone system
RANKL	Receptor activator of nuclear factor-κ B ligand
RCT	Randomized controlled trial
RR	Relative risk
SBP	Systolic blood pressure
SD	Standard deviation
SEVR	Subendocardial viability ratio
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor alpha
TTI	Tension time index
vitD	Vitamin D
vWF	von Willebrand factor
VCAM	Vascular cell adhesion molecule
VLDL	Very low density lipoprotein cholesterol
WHI	Women's Health Initiative
WHO	World Health Organisation

1.0 BACKGROUND

1.1 Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of death worldwide[1] and the second highest cause of mortality in Canada, both in men and women.[2] Atherosclerosis remains the most important pathophysiological mechanism underlying coronary artery disease and stroke[3], the two principal CVDs.[1] Modifiable risk factors of atherosclerosis include obesity, elevated blood pressure (BP), dyslipidemia, smoking, diabetes mellitus (DM), whereas some non-modifiable risk factors are age, sex, ethnicity, family history of atherosclerotic CVD, and genetics. New risk factors have emerged for atherosclerosis, including abnormal levels of lipoprotein-a, homocysteine, high sensitive C-reactive protein (hs-CRP), prothrombotic factors, and proinflammatory factors.[3-5] Among other emerging risk factors, intake of calcium,[6 7,8] has recently been reported to be associated with adverse cardiovascular (CV) outcomes. Importantly, about 90 % of CVDs are preventable through early identification of CV risk factors.[9] In recent years, health management guidelines have emphasized the need for close monitoring and early detection of CV risk factors, which can lead to more effective prevention of CVD.[1] Specifically, early detection of the atherosclerotic burden and the effect of risk factors on vascular damage even before the occurrence of CV events can be assessed non-invasively by measuring arterial stiffness and carotid intima-media thickness (cIMT),[5] specially in women.[10]

1.2 Cardiovascular Disease and Menopause

In general men are more predisposed to CVD than women.[11] However in women, loss of ovarian function and deficiency of natural estrogens following menopause increase the risk of CVD in postmenopausal compared to premenopausal women.[12,13,14]. Hence, female sex hormones, particularly estrogens, appear to have a protective effect against CVD.[12] In Canada, the prevalence of CVD increases from 3.5 % in women aged 45-64 years old to 14.8 % in those who are >65 years old.[11]

A meta-analysis reported increased risks of overall coronary heart disease (CHD) (relative risk [RR]:1.50; 95% confidence interval [CI]:1.28-1.76), fatal CHD (RR: 1.11; 95% CI: 1.03-1.20), and CVD mortality (RR: 1.19; 95% CI: 1.08-1.31) in women with onset of menopause <45 years old than in those with onset ≥45 years.[15] Similarly, prospective studies noted an increased risk of CHD,[16-19] CHD mortality,[20-22] as well as CVD mortality[23] in women with early menopause when compared to women with late menopause. Moreover, an increased risk of stroke in women with early menopause was reported in the Multi-ethnic Study of Atherosclerosis study (hazard ratio [HR]: 2.19; 95% CI:1.11-4.32 for menopause-onset at <46 years vs. ≥46 years)[16] and the Framingham study (HR:2.03; 95% CI:1.16-3.56; for menopause-onset at <42 years vs. ≥42 years)[24]. These results indicate that menopause is an independent risk factor of CVD and when attained early predisposes to increased prevalence of CVD than when attained later.

The increased risk of CVD in postmenopausal women can be partly explained by the increased incidence of CV risk factors[25,26] including the presence of metabolic syndrome,[27] elevated BP, central adiposity,[28,29] dyslipidemia,[30,31] insulin resistance[32] and inflammatory markers[33] as women transition from pre- to postmenopausal status. Moreover, increased cIMT, a surrogate marker of subclinical atherosclerosis[34] (discussed in section 1.6), was positively associated (p=0.02) with years since menopause in a cross-sectional analysis.[35]

1.2.1 Mechanisms of Increased Cardiovascular risk in Postmenopausal Women

Animal studies have given the most insight into the possible mechanisms implicated in increased CV risk in postmenopausal women. Specifically, loss of ovarian function and endogenous sex hormones deficiency were noted to activate the Renin-Angiotensin-Aldosterone system (RAAS)[36] through modulation by estrogen receptors,[37] resulting in endothelial dysfunction,[38] inflammation and immune dysfunction ultimately leading to elevated BP,[39] diastolic dysfunction and cardiac fibrosis.[37]

Earlier observational studies[40,41] reported favourable CV outcomes with estrogen replacement therapy or hormone replacement therapy leading to widespread postmenopausal usage. However, the results of the estrogen/progestin arm of the Women's Health Initiative (WHI) trial[42] demonstrated an increased risk of CVD with hormone treatment (HT), which has greatly limited the use of HT in postmenopausal women. These observations indicate that natural female sex hormones as compared to exogenous hormones are protective for CVDs in women.

1.3 Menopause, Osteoporosis and Calcium Metabolism

Osteoporosis is a skeletal disorder characterised by compromised bone strength as a result of reduced bone mass, as measured by bone mineral density (BMD), and deterioration of microarchitecture, predisposing to an increased risk of fracture.[43] The World Health Organization defined osteoporosis as a BMD of ≤ 2.5 standard deviations below that of young (30–40 year old), healthy adult women reference population, this is reported as a T-score.[44] Using this cutoff, the Canadian Multicenter Osteoporosis Study (CaMos) reported an approximate prevalence of osteoporosis of 9-12 % in Canadian women aged ≥50 years.[45]

1.3.1 Postmenopausal Osteoporosis

Bone mass is low at birth and increases over the next 2-3 decades of life to attain peak bone mass. During pubertal growth spurt, bone formation (by osteoblasts) exceeds bone resorption (by osteoclasts) leading to healthy bone remodeling. In adulthood and until middle age, bone resorption balances bone formation to maintain bone mass. However, after menopause, women experience reversal of bone loss because bone resorption exceeds bone formation.. This phenomenon is otherwise known as postmenopausal osteoporosis.[46]

With estrogen deficiency following menopause,[47] there is upregulation of cytokine production and function, thereby increasing osteoblastogenesis as well as osteoclastogenesis. However, estrogen

deficiency also favours a more rapid apoptosis of osteoblast as compared to osteoclasts, leading to more bone resorption than bone formation, and resulting in accelerated bone loss and reduced bone mass. [48] Moreover, estrogen suppresses receptor activator of nuclear factor-κ B ligand (RANKL), a promoter of osteoclast formation, differentiation, and survival,[49] as well as increases the expression of osteoprotegerin (OPG), a soluble decoy receptor for RANKL, leading to limited osteoclast development.[50] Hence, reduced estrogen concentration following menopause lead to increased RANKL concentrations and decreased OPG concentrations, resulting in increased osteoclastogenesis and accelerated bone loss. Furthermore, the cytokine suppressive effect of estrogen on interleukin (IL)-1,[51] IL-6,[52] tumor necrosis factor (TNF)-α[51,53] (responsible for bone resorption) is decreased following menopause. Drake et al.,[54] proposed that bone resorption leads to efflux of calcium from bone to extracellular fluid. Due to the increased calcium concentrations in the extracellular fluid, physiologic calcium homeostasis, through negative calcium balance, will decrease: a) renal calcium reabsorption,[55] b) intestinal calcium absorption[56] and, c) parathyroid hormone (PTH) secretion[57] thereby preventing the development of hypercalcemia. Estrogen replacement therapy can correct this negative calcium balance in postmenopausal women;[58] therefore showing that calcium metabolism is intricately related to osteoporosis development in postmenopausal women.

1.3.2 Calcium Metabolism

1.3.2.1 Distribution of Calcium

Ninety-nine percent of the total body calcium [1100 g (27.5 mol)] is distributed in the skeleton, whereas the remaining 1% stays in body fluids including extracellular fluid, intracellular fluid and plasma. The calcium in the body fluids exists in three forms: (1) as ionised calcium (Ca^{2+}) (about 50% of the calcium in the fluids), (2) bound to proteins (about 40% of the calcium in the fluids), and (3) complexed with other ions (about 10% of the calcium in the fluids). [59] Extracellular Ca^{2+} concentration is very tightly

regulated and is determined by the interplay of calcium absorption from the intestine, renal excretion of calcium, and bone uptake and release of calcium, each of which is controlled by vitamin D (vitD), PTH and calcitonin.[60] This precise control of calcium metabolism is very important as calcium plays an important role in contraction of muscles, blood coagulation, and nerve impulses transmission.[60]

1.3.2.2 Calcium Regulation by the Intestines and Kidneys

The usual calcium intake from diet is about 1000 mg/day. About 10% of net calcium intake reaches extracellular fluid following absorption through intestinal epithelium. Ca^{2+} ions in plasma get filtered through the glomeruli followed by a passage through the renal tubules, where 99% of the filtered calcium is reabsorbed, and 100 mg calcium/day is excreted in the urine.[60] Calcium absorption from the intestine is positively regulated by vitD, whereas reabsorption from the renal tubules is regulated by both vitD and PTH.[61]

1.3.2.3 Bone and Extracellular Calcium

About 30% of average compact bone is mainly constituted by organic matrix, primarily composed of collagen fibers. The rest (70%) consists of inorganic salts, principally calcium and phosphate. The major inorganic crystalline salt is hydroxyapatite [$Ca_{10}(PO4)_6(OH)_2$].

The bone calcium is in 2 different pools: an exchangeable pool, which rapidly exchanges about 500 mmol of Ca^{2+}/day between bone and plasma, and a much larger stable pool which is not readily exchangeable but dependent on the constant interplay of bone formation and resorption necessary for bone remodelling.[59] As previously mentioned, osteoblasts ensure bone formation, while osteoclasts are responsible for bone resorption through a control by RANKL, which is in turn produced by the osteoblasts and regulated by vitD and PTH, among others. Again, estrogen can affect both pathways, as it increases the production of OPG, a decoy receptor of RANKL, by osteoblasts, leading to bone resorption inhibition.[62] In adulthood and until middle age, the rate of bone formation is in equilibrium

with bone resorption, leading to constant total bone mass.[59] Calcium sensing receptors in the parathyroid gland control extracellular Ca^{2+} concentrations, and an imbalance will stimulate the release of PTH, a potent bone-resorbing agent,[62] and lead to either bone remodeling or vitD synthesis by the kidney in order to maintain calcium homeostasis.[63]

1.3.3 Calcium Intake and Osteoporosis

As calcium metabolism is intricately related to PTH and vitD metabolism, which is dependent on the balance between bone formation and bone resorption, adequate supply of calcium is important to ensure that bone laid down by osteoblasts is properly mineralized. This prevents bone loss and, hence calcium intake is often recommended to ensure optimal bone health.[64]

1.3.3.1 Recommendations and Average Calcium Intake

The age-specific Institute of Medicine recommendations of calcium intake (from diet or supplements) are: 1000 mg/day for 19-50 years (all); 1000 mg/day for 51-70 years (male); 1200 mg/day for 51-70 years (women); and 1200 mg/day for >70 years (all).[65] Osteoporosis Canada makes similar recommendations.[66] According to the 2003-2006 National Health and Nutrition Examination Survey (NHANES),[67] approximately 43% of the general population and almost 70% women >51 years age in United States relied upon supplemental calcium to have adequate amount of calcium. Similarly, the 2004 Canadian Community Health Survey [68] reported that 28% of men and 48% of women of ≥50 years age, were taking calcium supplements. The main sources of dietary calcium are milk and dairy products, whereas the two most common forms of supplemental calcium are calcium carbonate and calcium citrate.[69]

1.3.3.2 Calcium Intake and Prevention of Osteoporosis

A meta-analysis of randomized controlled trials (RCTs) studying the effect of calcium supplementation on bone health reported a 12% decreased risk of all fractures (RR: 0.88; 95% CI: 0.83-0.95), as well as

increase in BMD (RR: 0.54%; 95% CI: 0·35-0.73 at the hip, and RR: 1.19%; 95% CI: 0.76-1.61% in the spine), with the treatment effect showing better results with calcium doses ≥1200 mg than with doses <1200 mg.[70] However, the WHI trial (n=36282 women, age 50-79 years) did not report any reduction in the hip fracture incidence with intake of 1000 mg/day of elemental calcium supplementation for 7 years, although there was a small but significant improvement in hip bone density.[71]

Low habitual dietary calcium intake (dCa) was associated with an increased risk of fractures and osteoporosis in older women. Swedish mammography cohort study consisting of >60000 women, aged >40 years and followed up for 19 years, reported the highest rate of first fractures, as well as prevalence of osteoporosis in the lowest quintile of dCa, with an apparent decrease in the risk of any fracture, including hip fracture, and osteoporosis for every 300 mg increase.[72] However, two large meta-analyses of cohort studies did not find an association between milk intake and hip fracture risk.[73,74]

1.4 Calcium Intake and Cardiovascular Outcomes

Recently, meta-analyses[7,8] have shown that calcium supplements increase CV events, while other studies reported neutral or favourable outcomes.[75] On the other hand, while dCa has been thought generally to have a favourable effect on health,[76] recent studies reported a negative effect on CV health.[6,77] Therefore, the reports of adverse and beneficial health effects of calcium intake in the past few years have left the medical and scientific communities with much uncertainty.

1.4.1 Supplemental Calcium and Cardiovascular Outcomes

A meta-analysis of RCTs reported a 27-31% increased risk of myocardial infarction (MI), and non-significant increases in the risk of stroke (12-20%), a composite CV endpoint of MI, stroke or sudden death (12–18%) with calcium supplementation for at least 1 year.[7] Similarly, a 5-year RCT (The Auckland Calcium study) in healthy postmenopausal women noted a 66% increased risk of the composite endpoint of MI, stroke or sudden death with calcium supplement intake, which was driven by

an increase in the risk of MI (RR: 2.24; 95% CI: 1.20-4.17).[78] Likewise, prospective observational studies, such as the Kuopio Osteoporosis Risk factor and Prevention study[79] and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort study,[80] observed increased risk of CHD and CV events with calcium supplementation. An interaction between sex and calcium supplementation on CV mortality was reported in the Cancer Prevention Study II (Nutrition Cohort)[81] and National Institutes of Health (NIH)–American Association of Retired Persons (AARP) Diet and Health studies[82]: an increased CVD mortality with calcium supplementation was noted only in men in these studies.

However, another meta-analysis of 18 RCTs (n=63563) reported no significant associations of clinically verified CHD events (RR: 1.02; 95% CI: 0.96-1.09), MI (RR: 1.08; 95% CI: 0.92–1.26), angina pectoris and acute coronary syndrome (RR: 1.09; 95% CI: 0.95–1.24), chronic CHD (RR: 0.92; 95% CI: 0.73–1.15) with calcium supplementation.[75] Similarly, another meta-analysis of observational studies did not find any increased risk of CVD with calcium supplementation.[83,84] The very important WHI study, a RCT including more than 36000 postmenopausal women, did not observe any increase in adverse CV outcomes with daily 1000 mg elemental calcium carbonate and 400 IU vitamin D supplementation for 7 years.[85] Furthermore, a favourable effect on CV outcome was noted in Calcium Intake Fracture Outcome study (CAIFOS),[86] an RCT with 1460 postmenopausal women, where there was a significant 56% decrease in the risk CV events in those with pre-existing atherosclerotic CV disease following 1200 mg/d calcium supplementation for 5 years. Likewise, observational prospective studies like the Nurses' Health Study (NHS),[87,88] the Iowa Women's Health Study,[89] the Health Professionals Follow-up study[90] and the third National Health and Nutrition Examination Survey (NHANES III),[91] reported either favourable or neutral CV outcomes with calcium supplementation.

1.4.2 Dietary Calcium and Cardiovascular Outcomes

Most of the studies reported neutral or favourable effects on CV outcomes with dCa.

A recent meta-analysis of prospective studies did not find any association of dCa and CVD mortality; however, subgroup analysis reported a decreased risk of CVD mortality (pooled RR: 0.88; 95% CI: 0.78-0.99) in studies with a 10-year follow-up.[83] Similarly, no associations between dCa and CVD mortality were found in another meta-analysis of prospective cohort studies.[76] Furthermore, observational studies, such as the NIH-AARP study,[82] the NHANES-III study,[91] the Cancer Prevention Study (Nutrition Cohort)[81] and the Health Professionals Follow-up Study[90] did not report any association between dCa and CVD mortality. However, the EPIC-Heidelberg Cohort Study[80] reported a decreased risk of MI for the third quartile of dCa (HR: 0.69; 95% CI: 0.50-0.94) and dairy calcium (HR: 0.68; 0.50-0.93) when compared with the first quartile (820 mg/d and 466 mg/d vs. 513 mg/d and 188 mg/d, respectively).

The relationship with another CV event, stroke, with dCa has also been studied. The Japan Collaborative Cohort (JACC) Study,[92] the Japan Public Health Center (JPHC)-based study,[92] and the NHS[92] reported a decreased risk of mortality from or incidence of stroke with increasing dCa and dairy calcium intake. Similarly, subgroup analysis of a meta-analysis of prospective cohort studies reported a decreased risk of total stroke for those studies only, which included a follow-up period of >14 years (RR: 0.67 ; 95% CI: 0.51-0.88), as well as studies that investigated the association of dairy calcium (RR: 0.76; 0.66-0.86) with total stroke incidence.[77] Larson et al., in their meta-analysis, observed a dose-response association between dCa and risk of stroke: for every 300-mg/d increase of dCa, there was a significant 18% decreased risk of stroke for only those with dCa <700 mg/d; however, there was an increased stroke-risk (RR: 1.03; 95% CI: 1.01, 1.06) for those with habitual dCa >700 mg/d for the same increment of dCa.[93] The Swedish mammography cohort study,[6] which consisted of about 60000 women of >40 years of age

and who were followed for 19 years on average, did not observe an increase stroke risk with dCa; however, there was an increased risk of all-cause mortality (HR: 1.40; 95% CI: 1.17-1.67), driven by increased CVD mortality (HR: 1.49; 1.09-2.02), as well as ischemic heart disease (IHD) mortality (HR: 2.14; 1.48-3.09) for those with >1400 mg/d of dCa vs. 600-999 mg/d.

1.4.3 Total Calcium and Cardiovascular Outcomes

A meta-analysis of observational studies did not find any association between CVD mortality and total calcium intake, however, subgroup analysis reported higher CVD mortality for studies with a follow-up of >10 years (RR: 1.35; 95% CI: 1.09-1.68) in comparison to those with <10 years (RR: 0.88; 0.71-1.08).[83] The Swedish mammography cohort study[6] also had increased risk of CVD mortality (HR: 1.51; 95% CI: 1.23-1.84) and IHD mortality (HR=1.90; 1.45-2.49) in those taking >1400 mg/d vs. 600-999 mg/d. In the NIH-AARP study,[82] a men-only analysis revealed a U-shaped association between total CVD mortality and total calcium intake, with the highest risk noted for those taking ≥1500 mg/d. As compared to the lowest quintile (median=526 mg/d), the highest quintile (median=1530 mg/d) of total calcium intake was associated with a 12% significantly increased risk for total CVD mortality driven by an increased risk of CHD mortality (RR: 1.12; 95% CI: 1.04–1.21).

On the other hand, in the NHANES III, men-only analysis showed a significant 51% decreased risk of IHD deaths for those with total calcium intake of 1300–2000 mg/day vs. 1000–1300 mg/day.[91] Similarly, in a women-only analysis of the Cancer Prevention Study II (Nutrition Cohort)[91], there was a decreased risk for CVD mortality (RR: 0.81; 95% CI: 0.74-0.89) for the highest quintile of total calcium intake when compared to the lowest quintile. Likewise, in the Iowa women's health study,[94] there was a 33% decreased risk (RR: 0.67; 95% CI: 0.47-0.94) of death from IHD for the highest (>1425 mg/d) vs. the lowest (<696 mg/d) quartiles of total calcium intake. However, in the Health Professional Follow-up study[90] consisting of only men, there was no association between IHD and total calcium intake.

Although 2 studies from Japan, i.e. the JPHC-based study[95] and the JACC Study,[96] did not find any association between total calcium intake and risk of CHD, there was a decreased risk of *stroke* with increasing intake of total calcium. In the JPHC study,[95] there was a 29% decreased risk (HR: 0.71; 95% CI: 0.56-0.89) of total stroke in Japanese men and women when the highest (median=753 mg/d) quintile of total calcium intake was compared to the lowest (median=233 mg/d) quintile, which was primarily driven by a decreased risk of ischemic stroke (HR: 0.73; 95% CI: 0.53–1.02). Similarly, an earlier analysis of the Nurses' Health study (NHS)[92] (published in 1999) comprising of 85764 women followed up for 14 years, reported a decreased risk of ischemic stroke with increasing total calcium intake: those with highest quintile (median intake=1145 mg/d) had a 31% decreased risk (RR: 0.69; 95% CI: 0.50-0.95) of ischemic stroke when compared to the lowest quintile (median intake=395 mg/d) of total calcium intake.[92] However, a very recent analysis of the NHS comprising of the NHS I (n=86149 women) and NHS II (n=94715 women) cohorts did not report any significant association between total (RR: 1.04; 95% CI: 0.92-1.17) and ischemic (RR: 0.90; 95% CI: 0.76-1.08) stroke when the highest quintile of total calcium intake was compared with the lowest quintile.[88]

1.4.4 Possible Harmful Mechanisms Linking Calcium Intake and Cardiovascular Health

As a result of the inconsistent effect of calcium intake on CV outcomes, researchers have tried to elucidate the mechanisms that might underlie the adverse CV outcomes of calcium intake. The Auckland group proposed that the transient increased serum calcium following supplementary calcium intake was thought to be responsible for adverse health outcomes.[97] The rapid rise of serum calcium immediately following calcium supplementation, which was maintained even after 3 months of consumption,[98] was proposed to bear worse CV outcomes [99]. However, favourable changes in CV markers (a fall in Augmentation index (AIx), a marker of wave reflection[100] (discussed in 1.5.2.5), and a rise in subendocardial viability ratio (SEVR), an indicator of balance between myocardial oxygen

supply and demand[100] (discussed in 1.5.2.6) were observed acutely following an increase in serum calcium 3 hours after a single dose of 1 g of calcium citrate supplementation in healthy subjects, thereby contradicting the hypothesis that increased serum calcium induces adverse CV outcome.[101] Nevertheless, serum calcium has been linked to adverse CV and health outcomes,[102-104] as well as CV markers.[105] Serum calcium in healthy individuals is very tightly regulated,[106] and clinically important change of serum calcium is not expected following calcium intake. Hence, the observation of certain dose response relationships between calcium intake and health outcomes have led researchers to hypothesize that stringent homeostatic control of serum calcium by the interplay of PTH and vitD might be lost with extreme calcium intake (very low or very high intake).[6,76,91] However, this remains to be proven.

Calcium supplements were also thought to predispose to vascular calcification.[97] This was reported both acutely (i.e. 8 hours following 1 g/d calcium carbonate) and chronically (at 3 months) in an RCT.[107] Firstly, calcium supplements were reported to have a positive effect on activators of vascular calcification. Moreover, calcium supplements were noted to increase the propensity serum calcification: this was assessed by a novel method measuring the decline in transition time of conversion of primary, soluble calciprotein particles in serum to secondary, insoluble calciprotein particles, reflecting the relative concentrations of inhibitors and activators of calcification in serum.[107]

Calcium is also proposed to cause endothelial dysfunction, as well as activate the blood coagulation pathway, possibly leading to a deleterious effect on CV health.[97] Although not statistically significant, calcium supplementation increased blood coagulability over 8 hours following 1 g calcium citrate intake in postmenopausal women when compared to the placebo group.[108]

From the above evidence, it can be concluded that uncertainty also exists regarding the mechanisms underlying different CV outcomes with regards to calcium intake. Hence, the assessment of early

markers of vascular disease, including arterial stiffness and cIMT, would be ideal to examine the effect of calcium intake on vascular health at an early stage, even before the occurrence of any adverse outcomes.

1.5 Arterial Stiffness and Hemodynamics

1.5.1 Pathophysiology of Arterial Stiffness

1.5.1.1 Structure and Function of the Arterial System

Arteries are comprised of three distinct layers. From inner to outer, these are[109]:

1. Tunica intima: The innermost layer, consisting of a layer of endothelial cells surrounded by connective tissue, a basement membrane with elastic fibers;
2. Tunica media: The middle layer, consisting of concentric waves of smooth muscle cells along with elastin and collagen. This is the thickest layer;
3. Tunica adventitia: The outermost layer, formed of irregularly arranged collagen bundles, scattered fibroblasts, a few elastic fibers, and blood vessels.

There are intervening layers of elastic fiber between the tunica intima and tunica media, as well between the tunica media and tunica adventitia.

The proportion and structure of each layer varies with the size and function of the arterial segment in question, with the difference in compositions classifying the arteries into elastic and muscular arteries.[109] Compared to muscular arteries, the elastic aorta and large arteries consist of more elastin fibers and less smooth muscle cells, corresponding to their viscoelastic function. Specifically, this leads to dampening of the pulsatile output of the left ventricle, and transforming the intermittent and discontinuous activity of the cardiac pump into a continuous blood flow within the peripheral arteries, both in the systolic and diastolic phases of the cardiac cycle (Figure 1). In contrast, peripheral muscular

arteries, which contain more smooth muscle cells and less elastic fibers, are the site of peripheral arterial resistance.[110]

1.5.1.2 Mechanisms of Arterial Stiffness

Arterial stiffness represents the rigidity of the arteries. Arterial stiffening reflects an impairment in the viscoelastic properties of the vessels. The impaired viscoelastic property of the central arteries diminishes the beneficial buffering function of the aorta in systole (as described above), and leads to decreased organ and tissue perfusion in diastole (Figure 2).

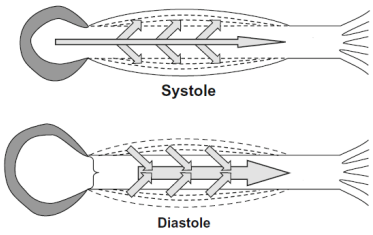


Figure 1 (Normal viscoelasticity)

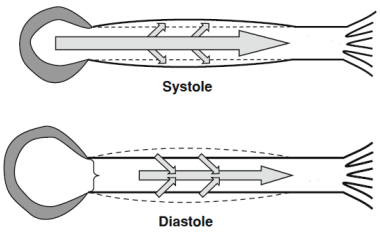


Figure 2 (Impaired viscoelasticity in arterial stiffening)

Modified from Salvi, P 2002[111]

The arterial viscoelastic property deteriorates as a result of changes to the structural and cellular elements of the arterial wall, as well as of the functional alteration of the arterial system. The relative imbalance of elastin and collagen, as well as endothelial dysfunction and change in the vascular smooth muscle tone can result in increased arterial stiffness.[112]

With ageing, elastin, which is responsible for the distensibility of the aortic wall, becomes gradually fragmented and degraded, while collagen content increases.[113,114] This change in the properties of elastin and collagen leads to more than a doubling of collagen concentrations as compared to elastin between ages 20-70 years.[110] Moreover, collagen is 100 to 1000 times stiffer than elastin.[115] Additionally, the fragmented elastin serves as a nidus for the microdeposition of calcium. Aging also brings about

increased cross-linking of collagen and elastin fibers by advanced glycation end products (AGE),[115] responsible for vascular stiffness.[116] Ultimately, these phenomena result in increased arterial wall stiffening with age.[117]

Inflammation related to ageing[118] and disease conditions[119] are also responsible for more imbalance between elastin and collagen.[114,120,121] Furthermore, physiologic hemodynamic alteration (as seen in elevated BP) through its differential effect on elastin and collagen[122] is also responsible for vascular stiffness: while elastin fibers are responsible for absorbing the load at low pressures, the load shifts towards the inelastic collagen fibers at higher pressures, thereby leading to increased stiffness of the arteries.[112]

Arterial stiffness is also affected by cellular components of the vessel wall, i.e. endothelial cells and vascular smooth muscle cells, as well as their function. Endothelial dysfunction, as evidenced by reduced nitric oxide (NO) expression, and increased expression of natural NO synthase inhibitor, has been linked to increased arterial stiffness.[123,124] Furthermore, arterial stiffening can be altered by vascular smooth muscle tone,[122] which can be modified by mediators such as angiotensin II, endothelin,[125,126] oxidant stress, and NO.[112]

Beyond aging, diseases, such as DM, atherosclerosis, chronic kidney disease, and smoking are associated with premature arterial stiffening, probably through their effect on the structural and functional properties of endothelial cells and vascular smooth muscle cells.[112]

1.5.2 Assessment of Arterial Stiffness

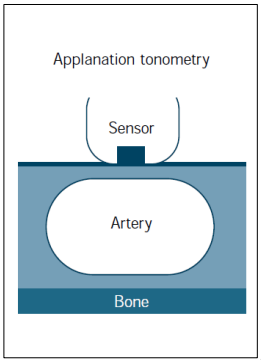
Several indices of arterial stiffness, including arterial distensibility, arterial compliance, stiffness index, Young's modulus, and elastic modulus, can be measured non-invasively by oscillometric, ultrasonographic, or magnetic resonance imaging (MRI) techniques, as well as with invasive techniques.[127] Oscillometric devices use a cuff surrounding the arm and/or ankle to capture pulse

waveforms from which arterial stiffness values are calculated. Ultrasonography and MRI calculate the stiffness indices by assessing the vessel distension and blood flow waveforms. The gold standard method for arterial stiffness measurement is recording the pulse wave by placing an intra-arterial catheter. However, this technique is also the most technically challenging method. One of the simplest ways of assessing arterial wall stiffness is through the measurement of pulse wave velocity (PWV) by applanation tonometry, a method both non-invasive and reproducible.[128]

1.5.2.1 Applanation Tonometry

Applanation tonometry involves placing a pen-like instrument with a micro sensor over the pulse to gently compress the artery against an underlying solid structure, such as a bone (Figure 3). This allows for the capture of a high-fidelity pulse waveform, and the measurement of PWV and pulse wave analysis (PWA). [129] In this thesis, the SphygmoCor system (AtCor Medical, Sydney, Australia), was used; this is based on the principle of applanation tonometry and it is validated both against invasive and non-invasive techniques (see 1.5.2.7).

Figure 3- Principles of Applanation Tonometry



Modified from the SphygmoCor user manual (AtCor Medical)[130]

1.5.2.2 Concept of Pulse Wave Velocity

PWV, a measure of arterial stiffness is inversely proportional to the viscoelastic property of the arterial wall, i.e. the less elastic the arterial wall, the higher the velocity, or PWV. PWV represents the speed at which the pulse pressure waveform travels from one site to another. It is calculated by measuring the

time the pulse wave takes to travel from the heart to a site relative to another site in the arterial tree, i.e. to a distal, peripheral site (radial/femoral artery) relative to a proximal site (common carotid artery). As velocity is equal to distance/time, then

$$PWV = \frac{\text{Distance between the two arterial segments}}{\Delta T};$$

where ΔT represents the time delay between pressure waveforms recorded in the distal segment relative to the proximal one, also known as pulse transit time (PTT). The two most common types of PWV are: carotid to femoral PWV (cfPWV) and carotid to radial PWV (crPWV).

Pulse Transit Time

The time delay between the proximal and distal pulse wave forms can be recorded with 2 transducers simultaneously placed at the 2 arterial sites to record the proximal and distal waveforms. The PTT can also be recorded at 2 different time points capturing the proximal and distal pulse waveforms with simultaneous electrocardiogram (ECG) recording, where the foot of the R wave of the QRS complex of the ECG is taken as a reference point. In this method, ΔT is calculated by: [(time between the foot of the R wave and the foot of the proximal pulse wave [T_1]) – (time between foot of the R wave and the foot of the distal pulse wave [T_2])] (Figure 4a). This latter method is utilised by SphygmoCor system.[130]

Measurement of the Distance between Proximal and Distal Arterial Sites

The distance between the proximal and distal sites can be assessed by a measuring tape using either the "direct" method, in which the distance between the proximal and distal sites is measured directly, or by the "subtraction" method, where the carotid-to-sternal notch distance is subtracted from the sternal notch-to-femoral (or radial) site distance (Figure 4b).[131] The cfPWV measured by the subtraction method approximates well the cfPWV recorded by MRI-based distance measurement,[132] as well as the gold standard invasive measurement.[133] Moreover, 80 % of the measured 'direct distance' between the carotid and femoral site (direct method) correlates well with the more accurate MRI-based method of

distance measurement.[131,134] Hence, the American Heart Association scientific statement on the 'Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness' advocates the use of the 'subtraction method' or multiplication of the direct distance between carotid and femoral sites by 0.8 to compute carotid to femoral distance for calculation of cfPWV noninvasively.[131]

Figure 4- Measurement of Pulse Wave Velocity by SphygmoCor

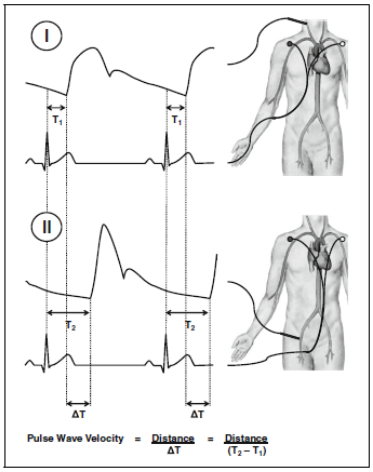


Figure 4a

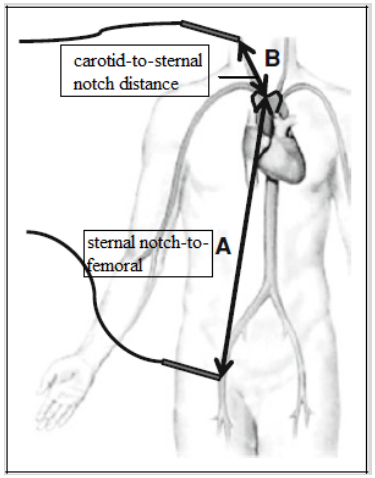


Figure 4b

Modified from Salvi, P 2002[111]

1.5.2.3 Clinical Relevance of Carotid to Femoral Pulse Wave Velocity

cfPWV represents the central arterial stiffness, whereas crPWV represents stiffness in peripheral arteries, and brachial to ankle PWV represents the stiffness of both central as well as peripheral arteries.[135]

Measurement of cfPWV is a more clinically relevant measure, as it represents the arterial segment that is most susceptible to stiffening as a result of aging and other risk factors.[128] cfPWV is also considered the 'gold standard' measurement for arterial stiffness.[128]

In 2010, the Reference Value for Arterial Stiffness Collaboration, using a standardized methodology in a large European population, provided normal and reference values for cfPWV.[136] For instance, the normal cfPWV value (mean±SD) for the age group of 50-69 years ranges from 7.6±1.4 to 10.3±2.4 m/s

for those who have optimal (systolic BP [SBP]/ diastolic BP [DBP]<120/80 mmHg), or normal (SBP/DBP=120-139/80-89 mmHg) BP, respectively. Importantly, these values increase with increasing BP levels. Furthermore, the Framingham Heart Study demonstrated a 48 % increased risk of a cardiovascular event with 1 SD increase in cfPWV, independent from individual vascular risk factors.[137] A meta-analysis noted an age-, sex-, and risk factor adjusted risk increase of 14%, 15%, and 15% in total CV events, CV mortality, and all-cause mortality, respectively, with a 1 m/s increase in cfPWV.[138] Hence, cfPWV is regarded as an independent predictor of CV outcomes.

1.5.2.4 Principles of Pulse Wave Reflection

When the heart contracts, a pressure wave propagates forward from the heart towards peripheral arterial sites. However, due to bifurcations of the vessels, this forward travelling wave gets reflected backwards to a certain extent. In normal conditions, the reflected wave returns to the central aorta in late-systole and early-diastole, and combines with the forward wave to form an augmented wave. The timing of superimposition of reflected wave on forward wave is very important as this increased amplitude of pulse wave helps to 'feed' the heart (myocardial perfusion) in the diastolic phase of the cardiac cycle. However, as the arteries stiffen, the reflected wave returns faster, leading to superimposition of the backward wave onto the forward wave early in systole. This results in increased central SBP (cSBP), increased central pulse pressure (cPP), increased ventricular load, as well as decreased myocardial perfusion in diastole.[139] Hence, increased cSBP and cPP are indicators of increased arterial stiffness.[127,131] Normally, while there is not much change in mean arterial BP (MAP) and DBP throughout the arterial system, cSBP and cPP are lower than brachial SBP and PP respectively, an occurrence termed 'amplification phenomenon'. As described before, with increased aortic stiffness, there is an increase of cPP without much change in peripheral PP (pPP), thereby resulting into a reduction in pulse pressure amplification (PPA). PPA is calculated by the ratio of pPP and cPP (PPA=

pPP/ cPP) and is another marker of vascular stiffening.[140,141] Aging and female sex are the two main non-modifiable risk factors leading to reduction in PPA,[141] whereas subjects with traditional CV risk factors like hypertension, DM, dyslipidemia, and smoking have been noted to have lower PPA independent of age and sex.[142] Interestingly, antihypertensive medications have varying effects on PPA: while β-blockers can reduce PPA, vasodilators such as angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blocker (ARB), calcium channel blockers (CCB), and nitrates increase PPA mainly as a result of their vasodilation property leading to reduction of pressure wave reflection.[143] These antihypertensive class-specific favourable effects on central hemodynamics were observed in the Conduit Artery Function Evaluation (CAFE) study[144] (a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]) and can also explain the difference in CV events reduction with the use of different BP-lowering drug regimens reported in the ASCOT trial, despite similar decrease in peripheral BPs (pBPs).[145]

1.5.2.5 Pulse Wave Analysis

Pulse wave analysis is based on the accurate recording of central BP waveforms (Figure 5a), and the specific analysis of their individual components.

The point on the central pulse wave where the forward and the reflected waves meet is called inflection point (Pi), and the time delay of the backward wave meeting the forward wave is represented by Tr. Lower value of Ti represents faster return of pulse wave, indicative of stiffening of the arteries. The peak of the systolic waveform represents the cSBP. The BP at the end of diastole represents the DBP. The difference between the cSBP and DBP is cPP. The magnitude of augmentation because of the superimposition of the reflected wave on forward wave as defined by (cSBP-Pi) is known as the augmentation pressure (AP).

Augmentation Index

AIx mainly represents overall wave reflection, and is defined as the ratio of AP and cPP, as shown by the following formula:

$$\textbf{AIx (\%)} = \textbf{(AP / cPP)} \times \textbf{100}$$

AIx is a 'continuum', ranging from positive to negative values, in relation to the timing of reflected waves.[111] If the Pi occurs after the peak systolic pressure in the pulse wave curve, then the value of AIx is negative and reflects the fact that central systolic pressure is not augmented as a result of the return of the reflected wave. On the other hand, a positive value of AIx indicates that Pi occurs before the peak systolic pressure due to the superimposition of the reflected wave early in systole leading to elevated central systolic pressure and ventricular load, and reduced myocardial perfusion in diastole (Figure 5a). As AP and AIx depend on the timing and magnitude of the wave reflection driven by arterial stiffness, they are also considered indirect measurements of systemic arterial stiffening.[111]

Heart rate is the most important factor affecting AIx, including arterial stiffness, vasomotor tone, magnitude and timing of reflected wave, structure of reflecting sites at peripheral circulation, as well as age, sex, and height of the subject. A 10-beats/minute increase in heart rate has been shown to reduce AIx by 3.9%.[146] Therefore, to negate the effect of heart rate, the SphygmoCor system calculates the AIx corrected for a heart rate of 75 beats/minute (AIx75) by applying this formula:

$$\textbf{AIx75} = \textbf{AIx} - \textbf{0.39*(75} - \textbf{Heart Rate)}$$

Studies have demonstrated that AIx is a marker of atherosclerotic burden[147] with high predictive value of CV events.[148-150] As several factors affect AIx, PWV is a better and more direct indicator of arterial stiffness in comparison to AIx.[111]

1.5.2.6 Subendocardial Viability Ratio

The SEVR represents the ratio between myocardial oxygen supply and demand. It can be derived noninvasively from the central pulse wave analysis (Figure 5b). The myocardial oxygen demand mainly depends on heart rate, ejection pressure, and myocardial contractility, and is represented by the systolic pressure–time index (SPTI) or the tension-time index (TTI) given by the area under the central pressure curve in systole. The myocardial oxygen supply depends on the subendocardial blood flow during diastole and is represented by the area under the curve of the central pressure curve in diastole, also known as the diastolic pressure–time index (DPTI). Hence, SEVR is given by:

DPTI/TTI

Low SEVR is associated with increased aortic stiffness,[151] as well as increased CV mortality[152] and morbidity as evidenced by increased severity of peripheral arterial disease,[153] and decreased coronary flow reserve resulting from a reduced SEVR.[154]

Figure 5- Pulse Wave Analysis (PWA) and PWA Indices

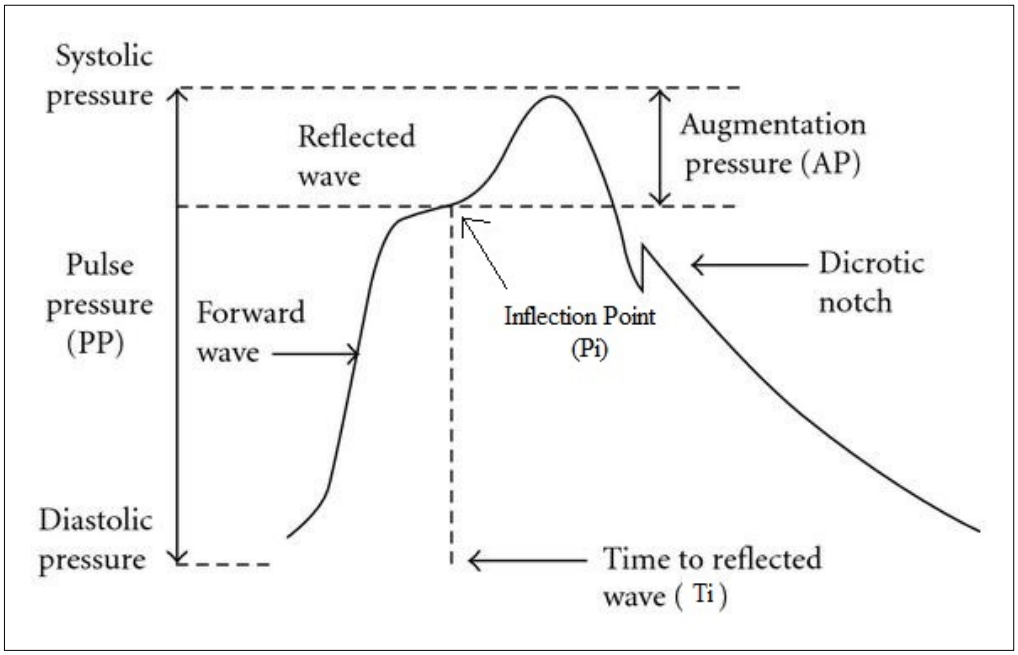


Figure 5a[155]

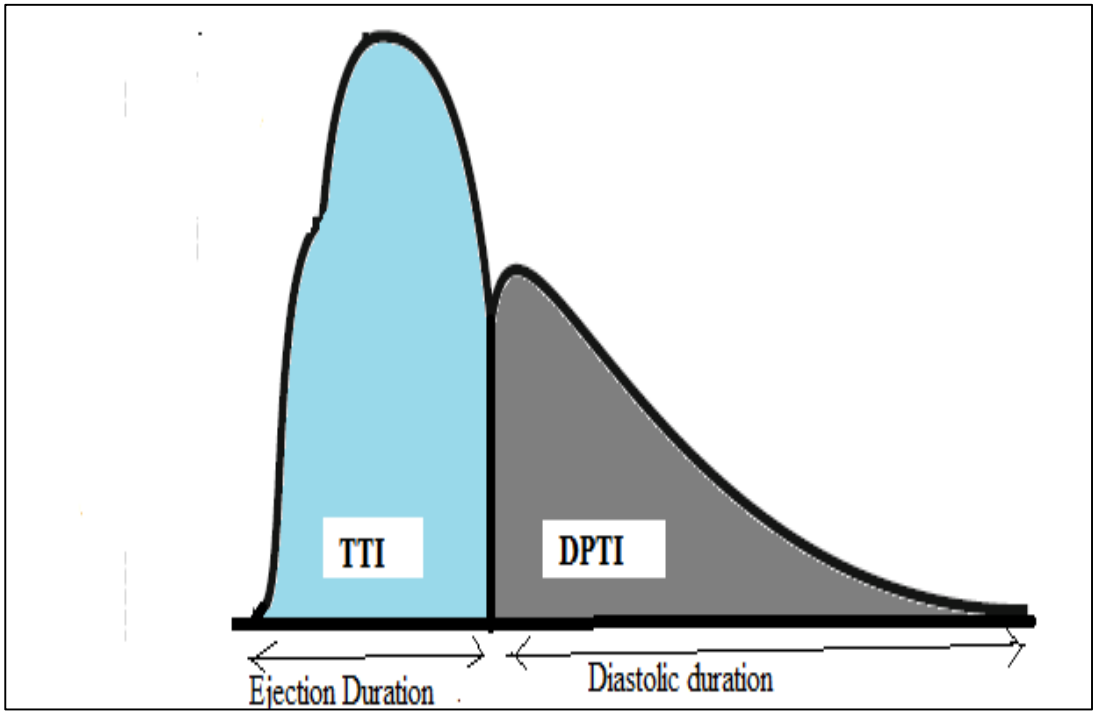


Figure 5b: Concept of Subendocardial Viability Ratio (Modified from Salvi, P 2002)[111]

1.5.2.7 Validity and Reproducibility

The validity of PWV (correlation coefficient [r]=0.73, $p < 0.0001$)[149] and central SBP ($r= 0.91$, $p < 0.001$)[5] obtained from SphygmoCor have been confirmed with gold standard direct invasive measurement of PWV and central BPs. Moreover, arterial stiffness measurements from SphygmoCor correlate well with similar indices obtained from other non-invasive methods[156-158] not only in healthy subjects, but also in diseased populations, such as with DM,[158] and in different conditions, including exercise and change of posture.[159-161] Furthermore, inter- (-0.3±1.25 m/s) and intra- (0.07±1.17 m/s) observer reproducibility of aortic PWV recorded by SphygmoCor was acceptable according to the 'ARTERY Society guidelines for validation of non-invasive hemodynamic measurement devices'.[162,163]

1.5.3 Calcium Intake and Arterial Stiffness Parameters

The association of short-term (acute effect), as well as long-term (chronic effect) of calcium intake and arterial stiffness has been reported in different studies.

Acute Effect

An interventional study in healthy subjects assessing the acute effect of calcium supplementation on arterial stiffness and central hemodynamics observed a significant decrease in AIx (from 29.7% to 26.4%, $p<0.05$), as well as a significant increase in SEVR (from 163% to 170%, $p<0.05$), without any significant change in cfPWV (8.2 m/s vs. 8.9 m/s, $p=0.33$), 3 hours following a single oral dose of 1000 mg calcium citrate when compared to baseline.[101] A cross-over RCT, comparing the acute effect of 600 mg of calcium either from dairy products or as calcium citrate on arterial stiffness reported no between-group difference in cfPWV following 2 hours of intervention, nor was there any difference in cfPWV between baseline and at 2 hours in within-group analysis.[164]

Chronic Effect

In a cross-sectional analysis of 587 men and women, there was a linear decrease (p-trend=0.018) in cfPWV (11.0 m/s for never/seldom dairy intake vs. 10.8 m/s for 1 time/week vs. 10.6 m/s for 2-4 times/week vs. 10.0 m/s for 5-6 times/week vs. 10.1m/s for ≥ once/d) with increasing dairy food consumption, with the principal source being dCa.[165] Similarly, in another cross-sectional study in Japanese men, dCa was found to be inversely associated with baPWV after adjusting for probable covariates (p-trend=0·02).[166]

From the above evidence, it remains uncertain whether calcium intake bears a beneficial or neutral effect on arterial stiffness.

1.6 Carotid Intima-media Thickness

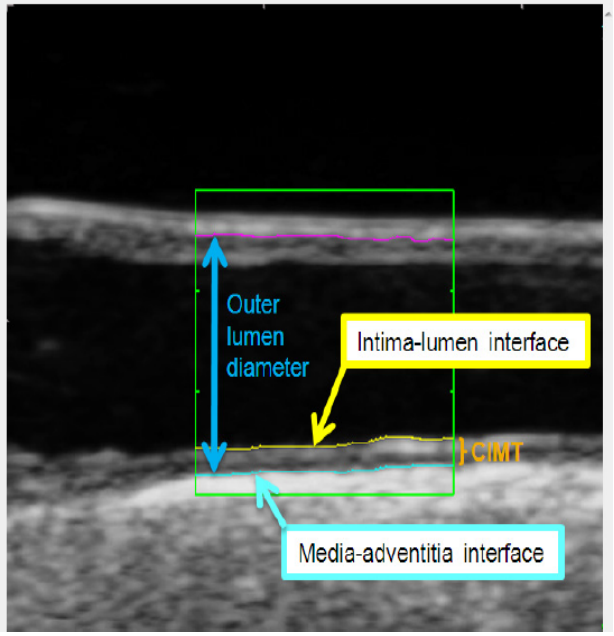
1.6.1 Anatomy and Histopathology of the Common Carotid Artery

1.6.1.1 Anatomy

The right common carotid artery (CCA) has its origin in the brachiocephalic trunk, while the left CCA arises directly from the arch of the aorta. Both CCAs bulge to form the carotid bulb at their distal end,

then bifurcate into external and internal carotid arteries to supply blood to the face and brain, respectively.[167] Similar to the structure of all arteries, the wall of the CCA consists of tunica intima (single layer of endothelial cells on a basement membrane), tunica media (layer of smooth muscle cells) and tunica adventitia (fibrous layer) from arterial lumen outwards.[109] The carotid intima-media thickness (cIMT) represents the combined width of the intimal and medial layers of the carotid arteries, which can be measured non-invasively by ultrasound examination. cIMT is measured by calculating the distance between the leading edges of lumen-intima and media-adventitia interfaces, visualized as the double line pattern on ultrasonographic examination of the CCA wall (Figure 6).

Figure 6- Measurement of Carotid intima-media thickness



Modified from Bauer M et al[168]

1.6.1.2 Histopathology

Atherosclerosis is one of the main pathophysiological mechanisms of CV disease.[3] Hypercholesterolemia and endothelial dysfunction lead to accumulation of low density lipoprotein cholesterol (LDL-C) and oxidized LDL (oxLDL) particles in the intimal layer of blood vessels.

Leukocyte migration, specially monocytes, also infiltrate the intimal layer, and engulf LDL particles, thereby forming foam cells, which further trigger inflammation, and exacerbate the progression of atherosclerosis. Furthermore, smooth-muscle cells from the adjacent tunica media layer migrate and accumulate within the expanding intima, resulting in extracellular matrix deposition, a process that forms the bulk of the atherosclerotic lesion.[169] Hence, the atherosclerotic process involves the intima-media complex of the artery, where increased thickness of intima-media layer represents an early stage, and atherosclerotic plaque formation is indicative of late stage of atherosclerosis, respectively.[3] In 1986, Pingoli et al. compared the CCA IMT measured by ultrasound examination (which they assessed as the 2 parallel echogenic lines [lumen-intima and media-adventitia interfaces] separated by a hypoechoic space [media]), with direct histological and microscopic examinations and reported no significant difference between the two measurements.[170] Subsequent ultrasonic-pathological comparison studies assessing the IMT also reported that the far wall rather than the near wall cIMT by B-mode ultrasound examination was better correlated with histological cIMT.[171,172] Moreover, the use of cIMT as CV marker is justified by the fact that cIMT correlates well with the atherosclerotic burden elsewhere in the arterial system.[173-178] However, easier site-accessibility (carotid artery) compared to other sites without any exposure to radiation makes cIMT examination by ultrasound a suitable clinical marker for CVD.

1.6.2 Carotid Intima-media Thickness and Cardiovascular Health

1.6.2.1 Carotid Intima-media Thickness and Cardiovascular Risk Factors

Studies have reported the association between cIMT and traditional CV risk factors. North American, and European population-based studies have reported a positive association between cIMT and age, as well as higher cIMT values for males compared to females.[173,179-186] Furthermore, similar to the racial variation in CVDs, there were ethnic differences in the values of cIMT: black subjects were found to have higher cIMT compared to South Asians and White populations, whereas the prevalence of carotid

plaque was higher in white subjects when compared to black subjects.[187,188] Moreover, other risk factors of CVD, such as hypertension,[189-192] DM and impaired glucose tolerance,[193-195] smoking,[190,196] obesity,[197,198] and dyslipidemia,[197-199] have been associated with increased cIMT in different populations. Hence, cIMT reflects several risk factors and can be regarded as a composite marker of CV health.

1.6.2.2 Carotid Intima-media Thickness and Cardiovascular Risk Assessment

Although the Framingham risk score (FRS)[200] is a useful tool to predict the risk of future CV events, it has certain disadvantages. The typical FRS predicts short-term risk (10-year risk) as opposed to lifetime risk; moreover, many major risk factors are not taken into account; and representation of some variables, such as smoking or DM, as categorical instead of continuous variables like level of blood glucose or smoke-year etc. also does not predict the actual risk.[180] These disadvantages have led to a rising dilemma regarding whether preventive therapies for CVDs are useful for those at intermediate CV risk (FRS 6-20 % without any established CVD). In this regard, the results of the Rotterdam study,[10] the *Atherosclerosis Risk in Communities* (ARIC) study,[201] and the *Carotid Intima Media Thickness and IMT-Progression as Predictors of Vascular Events in a High Risk European Population* (IMPROVE) study[202] support that cIMT may be a helpful tool in reclassifying those who are at intermediate CVD risk. Hence, the American College of Cardiology/American Heart Association (Class IIA) recommends cIMT assessments in individuals at intermediate CHD risk.[203] The National Cholesterol Education program Adult Treatment Panel III also proposed cIMT as a guide for intensification of lipid management.[204] The 2009 Spanish practice guidelines implementing the European Society of Hypertension/European Society of Cardiology recommendations have included cIMT to determine the presence of target organ damage induced by hypertension.[205]

1.6.2.3 Carotid Intima-media Thickness as Predictor of Cardiovascular Events

A meta-analysis (n= 37197) comprising 8 large, longitudinal population-based cohort studies showed that cIMT is a strong predictor of MI and stroke, with overall age- and sex-adjusted estimates of increased risk of MI and stroke of 26% and 18%, respectively, per SD increase of cIMT.[206] Similarly, the ARIC study,[207] the Cardiovascular Health Study (CHS),[181] the Rotterdam Study,[208] and the Carotid Atherosclerosis Progression Study (CAPS),[186] all prospective studies, also reported high predictive values of cIMT for CV events. In the ARIC Study, the adjusted HR of CHD was 5.07 (95% CI: 3.08-8.36) for women and 1.85 (1.28-2.69) for men for cIMT ≥1 mm vs. <1 mm when the participants were followed up for 4-7 years.[207] The CHS reported age-, sex- and other risk factors-adjusted RR of 1.27 (95% CI: 1.17-1.38) for stroke or MI per SD increase (1 SD=0.20 mm) in cIMT when the participants were followed up for 6.2 years.[181] Similarly, the Rotterdam study, with a follow-up of 2.7 years, noted age- and sex-adjusted odds ratios (ORs) of 1.57 (95% CI: 1.27-1.94) for stroke, and 1.51 (1.18-1.92) for MI, respectively.[208] Finally, CAPS observed adjusted HR of 1.16 (95% CI: 1.05–1.27) for MI and 1.17 (1.08–1.26) for the combined endpoint of MI, stroke or death per SD increase (1 SD=0.16 mm) in cIMT.[186]

1.6.2.4 Carotid Intima-media Thickness as a Therapeutic Target

As cIMT is a marker of subclinical atherosclerosis, the effect of CV risk modification interventions can be examined by assessing the change of cIMT over time.

The Measuring Effects on Intima-Media Thickness: an Evaluation of Rosuvastatin (METEOR) trial, the Pravastatin Lipids and Atherosclerosis in the Carotids (PLAC II) study,[209] the Regression Growth Evaluation Statin Study (REGRESS),[210] the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) trial,[211] the Asymptomatic Carotid Artery Progression Study (ACAPS),[212] and the Carotid Atherosclerosis Italian Ultrasound Study (CAIUS)[213] all evaluated the effect of different statins

on cIMT. They reported that statin therapy is associated with a slower progression of cIMT in different populations. β-blockers,[214] CCBs,[215] and ACEI[216] used for hypertension treatment were also reported to have a beneficial effect on cIMT. Similarly, optimal glycemic control in diabetic patients with anti-diabetic medications also resulted in a slower progression of cIMT.[217,218]

Hence, cIMT may be used to assess the efficacy of interventions aimed at CV risk reduction.

1.6.3 Reference/Normal Values of Carotid Intima-media Thickness

Based on age- and sex-specific cIMT values reported in different population studies,[173,179-185] ASE proposed that cIMT values ≥75th percentile are considered to be associated with high CVD risk, whereas values between the 25th-75th percentiles indicate unchanged CVD risk, and values ≤25th percentile suggest low CVD risk.[180] The Mannheim Carotid Intima-Media Thickness and Plaque Consensus also proposed reference cIMT values according to age group and traditional CV risk factors. For instance, the normal values of cIMT for the age groups 50-59 years and 60-69 years without any CV risk factor are: 0.70±0.05 and 0.75±0.05 mm, respectively. These values increase in the presence of CV risk factors.[219]

1.6.4 Calcium Intake and Carotid Intima-media Thickness

Few studies reported the effect of calcium intake on cIMT. Li et al. reported a significant increase in cIMT in postmenopausal women vs. premenopausal women with dyslipidemia [0.024±0.035 vs. 0.062±0.134 mm (p =0.003)] following 2 years of calcium supplementation. The authors also noted a significant interaction (p =0.017) between calcium supplementation and menopausal status on cIMT.[220] However, a sub-study of the Calcium Intake Fracture Outcome study reported no difference in cIMT [mean cIMT=0.778±0.006 mm vs. 0.783±0.006 mm (p=0.491); maximum cIMT=0.921±0.007 mm vs. 0.929±0.006 mm (p=0.404)] and carotid atherosclerosis [47.2% vs. 52.7% (p=0.066)] in postmenopausal women taking calcium supplements (CaS) or placebo. Nevertheless, a significant

reduction in carotid atherosclerosis was found in those postmenopausal women who maintained at least 80% compliance (CaS vs. placebo: 54.7 % vs. 46.7 %, p=0.033), as well as in participants in the highest tertile (>1795 mg/d) of total calcium intake vs. the lowest tertile (<1010 mg/d) (OR of carotid atherosclerosis: 0.70; 95%CI: 0.51–0.96). Therefore, it is remains inconclusive whether calcium intake has favourable, neutral, or adverse effects on cIMT.

1.7 Calcium Intake and other Cardiovascular Markers

1.7.1 Calcium Intake and Lipids

Hyperlipidemia is one of the most important risk factors for atherosclerotic CVD.[221]

Hypercholesterolemia leads to LDL and oxidized LDL particles build-up in the intimal layer of blood vessels, thereby triggering an inflammatory response, which in turn facilitates the migration of leucocytes into the arterial wall, and progression of atherosclerosis.[3] On the other hand, high-density lipoprotein cholesterol (HDL-C) is responsible for reverse transport of cholesterol, i.e. from tissues and cells to the liver, thereby leading to a favorable metabolic profile. Apolipoprotein (Apo)B is a protein constituent of the atherogenic lipoproteins, i.e. very low-density lipoprotein cholesterol (VLDL-C), intermediate density lipoprotein cholesterol (IDL-C) and LDL-C, while ApoA-I is the protein component of HDL-C. Thus, measurement of plasma ApoB and ApoA-I provides an assessment of the total number of atherogenic (LDL-C, VLDL-C, and IDL-C) and anti-atherogenic (HDL-C) particles, respectively.[222]

Supplemental as well as dietary calcium have been reported to have different effect on serum lipids, which has largely been dependent on study-design and population.

Calcium supplementation in RCTs of healthy subjects,[223] healthy postmenopausal women,[224] and IHD patients[225] led to favourable trends including significant improvement in lipid profile. It is proposed that the supplemented calcium interacts with fatty acids and bile acids in the gut to interfere with their

intestinal absorption, therefore increasing the fecal excretion of lipids.[226] Supplemental calcium might also have a beneficial effect on lipid profile through 1) favorable modulations of the gut environment for lactobacilli,[227] and 2) its effect on calcitropic hormones, i.e. suppression of circulating PTH and vitD, thereby promoting lipolysis.[228] However, in a study in dyslipidemic women, increased serum total cholesterol levels were noted in postmenopausal women, but not in premenopausal women, following 2 year of calcium supplementation.[220] This can be due to reduced cholesterol catabolism as a result of endogenous estrogen deficiency found in postmenopausal women.[229]

Dietary calcium mostly showed favorable effects on lipid profile. According to experimental evidence, dietary calcium has been noted to upregulate cholesterol catabolising enzymes and cholesterol transport from the enterocyte to the intestinal lumen, and downregulate cholesterol transport to enterocytes and the blood.[230] Furthermore, dietary calcium has also been reported to potentiate the positive effect of energy restriction on parameters of the metabolic syndrome: an energy restricted, high calcium diet for 4 months in obese subjects showed a beneficial effect on lipid profile.[231] However, similar calcium supplementation intervention with energy restricted diet for 3 months in another group of overweight subjects did not lead to any favourable effects on serum lipids, body weight, or waist circumference.[232] The fact that calcium-enriched dairy meals attenuated postprandial lipidemia, while calcium supplements did not, suggests that the chemical form of calcium or cofactors in dairy products might influence lipid metabolism.[233] Hence, the above evidence underlines the uncertainty as to whether calcium intake has favourable, neutral or adverse effects on serum lipids.

1.7.2 Calcium Intake and Blood Pressure

Hypertension increases the risk of CVD, including CHD, congestive heart failure, and stroke.[234,235] Essential hypertension contributes to 90-95% of all cases of hypertension. The pathophysiological basis of essential hypertension is a complex interaction of genetics and environmental factors, including high

salt intake, alcohol consumption, obesity and weight gain, psychosocial stress, and low levels of physical activity. The other 5-10 % of hypertensive cases is termed secondary hypertension where hypertensive patients have increased BP because of endocrine diseases, kidney diseases, or medications, among others.[236,237] Many studies have examined the relationship between calcium intake and BP.

A meta-analysis of 40 RCTs reported that calcium supplementation (mean dose: 1200 mg/day), when compared to the non-supplemented group, reduced SBP by 1.86 mmHg (95% CI: -2.91- -0.81), and DBP by 0.99 mmHg (-1.61- -0.37). The effect was exacerbated in those with a low habitual calcium intake (≤800 mg/day): -2.63 mmHg (95% CI: -4.03- -1.24) for SBP, and -1.30 mmHg (-2.13- -0.47) for DBP, respectively.[238] A previous meta-analysis also reported similar findings with calcium supplementation when compared to placebo (SBP change: -1.44 mmHg, 95% CI: -2.20- -0.68; DBP change: -0.84 mmHg, 95% CI: -1.44- -0.24]).[239] Another meta-analysis particularly focused on hypertensive individuals, and reported that the calcium supplemented group, as compared to the non-supplemented group, had a significant reduction in SBP (mean difference: -2.5 mmHg; 95% CI: -4.5- -0.6) without a significant change in DBP (mean difference: -0.8 mmHg; -2.1-0.4).[240] Although most RCTs[241,242] reported an inverse association with BP, the WHI trial,[243] which followed more than 36000 postmenopausal women taking 1000 mg of elemental calcium and 400 IU of vitD3 or placebo daily for >7 years, did not report a significant association between calcium supplementation and change in BP (change in supplementation vs. non-supplementation groups: SBP: 0.22 mmHg; 95% CI: -0.05 - 0.49 mmHg and DBP=0.11 mmHg; -0.04 - 0.27 mmHg).

Dairy food, the major source of dietary calcium, has been reported in a meta-analysis to bear an inverse association (RR: 0.87; 95% CI 0.81-0.94) with BP, which was more pronounced with low-fat dairy food (RR: 0.84; 0.74–0.95).[244] Similarly, subgroup analyses of another meta-analysis reported an inverse association between dCa and BP, noting a significant reduction of SBP and DBP of 0.010 mmHg ($p <$

0.001) and 0.009 mmHg ($p < 0.05$) in men and, 0.15 mmHg ($p < 0.001$) and 0.057 mmHg ($p < 0.02$) in women, respectively, for every 100 mg increase in dietary calcium.[245] These results support that increased dietary intake of calcium may be associated with lower BP.[246-248]

The regulation of BP by calcium intake is proposed to be mediated by the natriuretic effect of calcium,[69] in addition to its regulation of vascular smooth muscle tone through the modulation of vitD or PTH metabolism,[249] as well as by affecting RAS,[250] and reducing salt sensitivity.[249]

Although most studies report a reduction of BP with calcium intake, more research is required to assess whether this favourable association has any effect on CV outcomes.

1.7.3 Calcium Intake and Vascular Biomarkers

Atherosclerosis and inflammation are intricately related: inflammation bears the link between CV risk factors and the altered arterial biology seen in atherosclerosis. CV risk factors lead to endothelial dysfunction, which is characterized by an increased expression of cell adhesion molecules (CAM), such as vascular CAM (VCAM), and intracellular CAM (ICAM) that help in recruitment of inflammatory monocytes/macrophages and T-lymphocytes which release inflammatory cytokines (e.g. IL-1, IL-6, TNF-α). Inflammatory cytokines in turn drive the formation, progression, and rupture of atherosclerotic plaques, as well as stimulate the formation of acute phase reactants, such as C-reactive protein (CRP) from the liver, thereby perpetuating more inflammation. Therefore, not only intravascular inflammation, but also any cause of high inflammatory burden including extravascular causes have been implicated in the pathogenesis of atherosclerosis.[251] Thus, increased levels of cytokines not only reflect the inflammatory status of an individual, but also the atherosclerotic burden or the degree of inflammatory activity within the atheromas.[169] This has been proven by prospective studies where assessment of serum inflammatory markers are associated with increased risk of CVD.[252] Furthermore, recent evidence from the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin

(JUPITER) trial[253] supports the use of inflammatory markers as a guide to therapy for reduction of CV events in apparently healthy people without any traditional CV risk factors.

Thrombosis is a complication of atherosclerosis mainly mediated by factors, such as von Willebrand factor (vWF) and fibrinogen. vWF helps in platelet adhesion on the injured endothelium, the first step in thrombus formation, whereas fibrinogen helps in platelet aggregation, thereby mediating progression of thrombosis.[222] Both these vascular biomarkers have been noted to be associated with atherosclerotic CVD, and highly predictive of CVD mortality.[254-257]

The effect of calcium intake on vascular biomarkers to assess the relationship between calcium and CV health has been reported in many studies and in varied populations. The studies for the relationship between dCa and vascular biomarkers mostly concentrated on milk and dairy products. In a cross-sectional study, low fat dairy food consumption was inversely associated with CRP, IL-6 and soluble VCAM-1 in healthy subjects.[258] Similarly, in an RCT which included overweight and obese subjects, there was a significant fall in inflammatory markers (TNF-α, IL-6, monocyte chemoattractant protein-1 [MCP-1]) after 28 days of dairy diet (consisting of 170 kcal, 10 g protein, 1 g fat, and 30 g carbohydrate) taken 3 times/day, when compared with baseline.[259] Even in smokers, there was a fall in inflammatory markers (TNF-α, IL-1, and IL-6) following 6 weeks of milk intake when compared to baseline.[260] Moreover, there was an acute fall of inflammatory markers (TNF-α, IL-1β, and IL-6), as well as of other vascular biomarkers (VCAM, MCP-1, and macrophage inflammatory protein -1) only after 3 hours of a single dairy meal in overweight subjects.[261] The Attica study from Greece further reported an inverse association between inflammatory markers (TNF-α, IL-6 and, CRP) and increasing number of weekly servings of dairy products.[262] However, other interventional studies reported no significant change in vascular biomarkers following dairy interventions in healthy, and overweight subjects.[261,263]

Calcium supplementation on the other hand has been mostly reported to bear no relationship with inflammatory or vascular biomarkers. A RCT in healthy postmenopausal women receiving either 1000 mg of elemental calcium or placebo for 1 year reported no difference in CRP levels between the two groups.[264] Similarly, RCTs in participants with impaired fasting blood glucose,[265] as well as patients with type 2 DM (T2DM) [266] did not report any significant change in inflammatory markers following calcium supplementation. However, another study in patients with T2DM, calcium fortified yogurt for 12 weeks resulted in decreased levels of high sensitivity CRP (hs-CRP), IL-1, IL-6, and fibrinogen.[267] Ultimately, the evidence presented above indicates that the effect of calcium intake on inflammatory as well as vascular biomarkers is largely inconclusive, and further research is needed.

2.0 OBJECTIVES & HYPOTHESIS

The overall aim of this thesis is to examine the effect of dCa on CV health in healthy postmenopausal women. Specifically, this thesis examines the association of dCa and the following established CV markers: 1) cIMT, 2) arterial stiffness and hemodynamics and 3) serum lipids. Our hypothesis is that dCa is not associated with increased CV risk.

3.0 METHODS

3.1 Study Design

This study is part of a larger Canadian Institute of Health Research (CIHR)-funded randomized controlled trial (**Calcium Study**: "The effect of dietary calcium intake as compared to calcium supplementation on vascular and bone health in postmenopausal women", ClinicalTrials.gov NCT01731340), in which study participants are assessed on 3 occasions: at study entry (baseline assessment), at 6 months, and at 12 months after the baseline assessment. This thesis comprises of the *cross-sectional analysis of baseline data* of the Calcium Study participants.

The study was approved by the research ethics and scientific review boards of the McGill University Health Centre (MUHC) and conforms to the standards set by the previous and most current version of the Declaration of Helsinki.[268] The vascular assessments were conducted at the Vascular Health Unit (Montreal General Hospital, MUHC).

3.2 Research Subjects

3.2.1 Recruitment & Screening

Recruitment for the Calcium Study was done through advertisement on McGill University and Greater Montreal area websites, social media, newspapers, and magazines, in both French and English (Appendix A).

Screening

Interested applicants were screened for eligibility firstly over the telephone. Upon meeting the eligibility criteria, participants were invited to an on-site visit for confirmation of inclusion and exclusion criteria (Appendix B).

3.2.2 Study population

3.2.2.1 Inclusion Criteria

Postmenopausal women are at increased risk of osteoporosis compared to premenopausal women or men of similar age[269] and hence, Health Canada recommends they have adequate amount of calcium intake.[270] More importantly, women following menopause are also at increased risk of CVD. Thus, postmenopausal women were selected as the study group as an association between calcium intake and markers of CVD can be evaluated more comprehensively in postmenopausal women than any other age-group population.

Healthy, postmenopausal women (50 years and older, at least 3 years since last menstrual period) without any chronic disease and from the greater Montreal area were included in the Calcium study.

3.2.2.2 Exclusion Criteria

Women with established atherosclerotic CVD, body mass index (BMI) <19 kg/m^2 and >35 kg/m^2, types 1 and 2 DM, hyperparathyroidism, treated hypertension or with a SBP>140 mmHg and/or DBP>90 mmHg, history of gestational DM, history of gestational hypertension or pre-eclampsia, history of smoking (any type) in the past 5 years, history of urinary tract lithiasis, history of bone active agents use in the previous 3 years prior to randomization, history of calcium or vitD supplementary use in the 2 months prior to randomization, and those who have a high (>20%) 10-year absolute risk of major osteoporotic fractures (calculated using the fracture assessment tool FRAX without BMD)[271] were excluded from the study.

3.3 Assessment Day

Upon inclusion in the study, the participants completed an interviewer-administered questionnaire, including information on socio-demographic status, past personal and family medical history of CVD and bone disease, medication and supplement use, and lifestyle habits.

Participants were further asked to abstain from vigorous physical activity and alcohol consumption for 48 h and fast overnight for 12 h before their visit.

Written informed consent was obtained from all participants (MUHC Research Ethics Board #GEN-11-231).

3.3.1 Assessment of Dietary Calcium Intake

Usual dCa in the previous month was assessed in all participants by a research dietitian using a validated 51-item food frequency questionnaire (Appendix D).[272] Food models were used to evaluate the nutrient intake and estimate of intake was assessed by portion sizes.

3.3.2 Assessment of Physical Activity

The International Physical Activity Questionnaire (IPAQ)[273,274] was used to estimate physical activity status where the participants were asked to report the time they spent performing specific types of physical activities in the previous 7 days (Appendix C). Metabolic equivalents were calculated based on the activity type as per the IPAQ guidelines.[275]

3.3.3 Anthropometric Measurements

Height, weight, and waist and hip circumference measurements were performed using standardized protocols. Standing height was measured by a wall-mounted steadiometer (Seca 242, Hamburg, Germany),[276] while weight was measured in light clothing without wearing shoes (Tanita TBF-310; Tanita Corp., Tokyo, Japan).[277] Waist and hip circumferences were measured according to the NIH guidelines (Appendix E).[278]

3.3.4 Vascular Studies

All measurements were performed under standardized conditions in a quiet, controlled environment with room temperature at 22±1°C and humidity 60±5%, in the morning (Appendix E)**.**

3.3.4.1 Resting Blood Pressure Assessment

After a 10 minutes rest, peripharal SBP (pSBP) and DBP (pDBP) and heart rate were assessed with the automated oscillometric BpTRU device (BpTRU Medical Devices Inc., Coquitlam, BC, Canada), in accordance with the Hypertension Canada guidelines.[279]

3.3.4.2 Carotid Intima-media Thickness Measurement

The cIMT measurements were obtained with a Philips iU22 Vision 2011 xMATRIX ultrasound system (L9-3 probe). Both the technician and the participant were positioned properly according to a standardized protocol. Briefly, the subject should lie down with their head rotated 45° in the direction opposite to the side (right/left) examined.[14]

Transverse B-mode ultrasound scanning from proximal CCA to middle of the internal CA was first performed to get an overview of vessel anatomy and presence of any plaque. This was followed by the visualization of the near and far wall of the CCA and carotid bifurcation (bulb) in longitudinal view in 3 different projections (anterior, lateral, and posterior) (Figure 7). Cine loops as well as single-frame still images of CCA centered 20 mm proximal to the carotid bulb were obtained in a plaque-free region. Cine loops were obtained at a frame rate of 32 per second in an appropriate depth of focus (30–40 mm), with suitable gain settings to eliminate intraluminal artifacts. Simultaneous 3-lead ECG helped to analyze end-diastolic cIMT images automatically (by QLab, Philips automated IMT calculation software) over a length of 10 mm on the far wall of CCA. cIMT, visualized as a double line pattern, is the distance between the leading edges of lumen-intima and media-adventitia interfaces (Figure 8). Carotid plaque is defined ***either*** a) by an increase of cIMT of ≥0.5 mm or an increase of cIMT by about 50%, compared with the adjacent cIMT, ***or*** b) when cIMT is >1.5 mm.[219] The maximum cIMT measurements from the left and from the right CCA were selected for further statistical analysis.

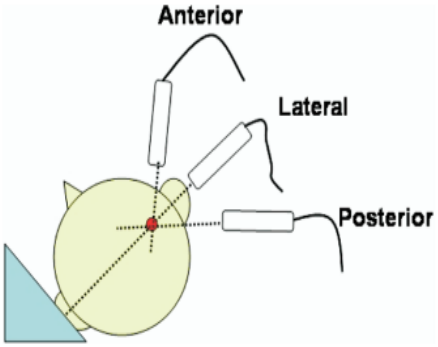


Figure 7 (Modified from Stein JH et al.[14])

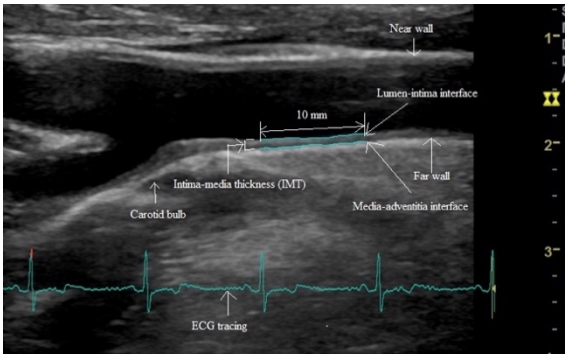


Figure 8 (Modified from Ho SS et al.[280])

3.3.4.3 Arterial Stiffness and Hemodynamic Measurements

Arterial stiffness and hemodynamic measurements were obtained in duplicates via applanation tonometry, using a validated SphygmoCor system (AtCor Medical, Sydney, Australia). A high-fidelity hand-held tonometer (SPC-301; Millar Instruments) placed over the arteries (carotid, femoral, or radial) was used to record the pulse waves.

For calculation of PWV, a simultaneous 3-lead ECG helped measure pulse transit time using the 'foot-to-foot' method. The PTT was calculated by subtracting the time difference between the foot of R-wave of ECG and the foot of distal pulse (femoral or radial artery) from the time gap and between the foot of R-wave of ECG and the foot of proximal pulse (carotid artery) (see 1.5.2.2). The distance measurement was performed by the 'subtraction method', as the subtraction of the carotid-to-sternal notch distance from the sternal notch-to-femoral (or radial) site distance (see 1.5.2.2). The SphygmoCor system calculates the PWV as the ratio of distance measured and PTT. The PWV measurements with ECG-individual site (carotid, femoral or radial) PTT SD>5 % as well as overall femoral (or radial)-carotid PTT SD>10 % were discarded. Duplicate PWV measurement values with a difference of ≤0.5 m/s were selected to minimize variability between measurements as per recommendations.[131]

For PWA (arterial hemodynamics), the SphygmoCor system utilizes a generalized transfer function to obtain central pulse waveform from the radial pulse wave recorded by applanation tonometry. The central hemodynamic parameters (cSBP, cDBP, MAP, cPP, PPA, AP, AIx, AIx75 and, SEVR) were obtained in duplicates from the central PWA after calibrating for pBPs. All PWA measurements were evaluated by SphymoCor's internal quality system and only those with operator index ≥ 85 %, and ≤ 4 units of AP, AIx, and AIx75 were selected.

3.3.5 Assessment of Biomarkers

Morning fasting blood samples were collected. The blood samples were first separated into plasma component following centrifugation of 3000 revolutions/minute for 10 minutes at 4° centigrade. Following this, a part of the plasma was used for extraction of serum fraction after a second centrifugation of 3000 revolutions per minutes for 10 minutes at 4° centigrade. Finally, the plasma and serum fractions were stored at −80°C for further analysis at a later date at McGill University Health Centre Clinical Laboratories, Glen site. Measurements of hsCRP and serum lipids, including total cholesterol, triglycerides (TAG), HDL-C, and, apo-B, were performed using Synchron LX Systems (Beckman Coulter Inc.). The intra-assay % Coefficient of Variation (CV) for hs-CRP, total cholesterol, TAG, HDL-C and apo-B were: 0.9 %, 0.7 %, 0.6 %, 1.5 %, and 0.9 %, respectively. LDL-C was calculated using the Friedewald equation: LDL-C= (Total cholesterol- [HDL-C+ TAG/5]) in mg/dL.[281] Plasma 25-Hydroxy vitD [25(OH)D] concentration was measured using the Laison Total 25(OH)D assay (DiaSorin Inc, Stillwater, MN, USA) at McGill University at the School of Dietetics and Human Nutrition (McDonald Campus, Sainte-Anne-de-Bellevue, Quebec, Canada). The intra-assay and inter-assay CVs were <5%, and the accuracy was 91-93% for National Institutes of Standards and Technology Standard Reference Material (972a, Level 2) (vitD Metabolites in Frozen Human Serum).

3.4 Statistical Analyses

Summary statistics for baseline characteristics of the study participants were presented as mean and standard deviation (SD), or median and inter-quartile ranges (IQR), as appropriate, for continuous variables, or as counts and percentages for categorical variables. Analysis of normality for continuous variables was performed using the Kolmogorov-Smirnov test.

One-way analysis of variance (ANOVA, for continuous variables) or Chi-squared test (for categorical variables) was performed to compare the demographic characteristics, vascular measurements (including cIMT, PWVs, hemodynamic parameters, and serum lipids) across groups of <600, 600-1000, and >1000 mg/daily consumption of dCa, respectively. These cutoffs of dCa were selected according to Canada's Food guide recommendations for women >50 years, which recommend three servings of milk and dairy products/day, where each serving usually consists of about 300 mg of elemental calcium.[282,283] Analysis of covariance (ANCOVA) was also performed after controlling for covariates, to compare vascular measurements and serum lipids, across the same groups of dCa. The covariates chosen for the ANCOVA analysis were age, BMI, history of smoking, MAP and dvitD, as these variables had significant correlation with the dependent variables vascular and lipid parameters. Furthermore, we conducted an exploratory analysis followed by a subgroup analysis of 600-1000 mg/d dCa group as the participants in this group had more favourable values of vascular markers than the rest of our population.

The value of statistical significance was set at $p<0{\cdot}05$. Statistical analyses were performed with SPSS version 22 (IBM Corp. Armonk, NY).

4.0 RESULTS

The vascular parameters including cIMT, PWV, and hemodynamic parameters were analysed in 96 participants (Main Study). Additionally, analyses for biochemical markers including serum levels of 25(OH)D, hsCRP, and serum lipids were performed separately as they were available in a subgroup of 80 participants (Secondary Study).

4.1 Analyses of Vascular Parameters (Main Study)

4.1.1 Participant Characteristics

Participant characteristics (n=96) are presented in **Table 1.1**. The mean±SD values of age, BMI and waist-hip ratio (WHR) were: 60.2±6.3 years, 25.6±3.9 kg/m^2, and 0.88±0.06, respectively. Although none of the participants were current smokers, there was approximately equal distribution between former-smoker and never-smoker (51 % and 49 %, respectively, $p>0.05$). Their average values of dCa and dvitD intakes were 859±353 mg/d (mean±SD), and 221 (154, 296) IU/d (median [IQR]), respectively. The median (IQR) values of cIMT and cfPWV for all participants were 0.63 (0.57, 0.69) mm, and 7.3 (6.4, 8.1) m/s, respectively. Their average values of pSBP and pDBP were 113±11 mmHg (mean±SD), and 72 (69, 76) mmHg (median [IQR]), respectively.

4.1.2 General Characteristics of Participants across Dietary Calcium Intake Groups

Participant characteristics were examined across dCa intake groups: 1) <600 mg/d, 2) 600-1000 mg/d, and 3) >1000 mg/d. Results are presented in **Table 1.2.1**. The mean±SD dCa intake in groups 1, 2, and 3 were 466±94, 801±119, and 1300±241 mg/d, respectively. The mean age of the participants in group 3 was significantly higher than group 1 ($p=0.02$), without any other significant between-group difference in age. There was no significant difference in BMI among the 3 groups of dCa. However, the number of former smokers was significantly lower in group 3 compared to groups 1 and 2 ($p=0.02$), without any other inter-group significant difference for the history of smoking. The median intake of dvitD

proportionately increased from group 1 to 3, and was significantly higher in group 3 compared to groups 1 ($p<0.001$) and 2 ($p<0.001$), and in group 2 than group 1 ($p=0.009$), respectively. In unadjusted analysis, there was no significant differences in the vascular parameters, including cIMT, cfPWV, and other hemodynamic parameters among the 3 groups of dCa, presented in **Table 1.2.2.**

4.1.3 Vascular Parameters in Dietary Calcium Intake Groups

The vascular parameters, including cIMT and cfPWV, and hemodynamic parameters, were compared across the dCa intake groups: 1) <600 mg/d, 2) 600-1000 mg/d, and 3) >1000 mg/d after adjustment of the following covariates: age, BMI, history of smoking, MAP and dvitD, as presented in **Table 1.3**.

cIMT. The cIMT value was numerically higher in group 3 compared to groups 1 and 2; however, there was no significant difference among the three groups. The adjusted mean value of cIMT in group 3 was 0.04 mm higher than groups 1 and 2.

cfPWV. Although there was no significant difference in cfPWV among the three groups, cfPWV was numerically higher in group 3 compared to groups 1 and 2. The adjusted mean value of cfPWV in group 3 was 0.5 m/s higher than groups 1 and 2.

crPWV. The crPWV was numerically higher in group 3 compared to groups 1 and 2; however, there was no significant difference among the three groups. The adjusted mean value of crPWV in group 3 was 0.4 m/s higher than groups 1 and 2.

Peripheral hemodynamic parameters. pSBP and pDBP were numerically lower in group 2 compared to groups 1 and 3, although the difference among the three groups was not statistically significant. The adjusted mean values of pSBP and pDBP were numerically 2-3 mmHg and 1-2 mmHg lower in group 2 compared to groups 1 and 3, respectively.

Central hemodynamic parameters. The central hemodynamic parameters including cSBP, cPP, and MAP were higher in group 3 compared to groups 1 and 2; however, there was no significant difference

among the groups. The adjusted mean values of cSBP, cPP, and MAP were numerically 2-5 mmHg, 3 mmHg, and 2-3 mmHg higher in group 3 compared to groups 1 and 2, respectively.

Although there was no significant difference in the value of cDBP among the 3 groups, cDBP in group 2 was the lowest among the 3 groups: the adjusted mean value of cDBP in group 2 was 1-2 mmHg lower compared to groups 1 and 3, respectively.

The adjusted mean value of PPA in group 2 was numerically 0.02-0.03 higher compared to groups 1 and 3, showing a non-significant reverse U-shaped relationship between dCa groups and PPA.

Although no difference in AP was observed among the 3 groups of dCa in adjusted analysis, a U-shaped relationship between dCa groups and AIx75 was observed: the adjusted mean value of AIx75 value in group 2 was numerically 3-4% lower compared to groups 1 and 3.

SEVR was numerically lower in group 3 compared to groups 1 and 2; however, there was no significant difference among the three groups. The adjusted mean value of SEVR in group 3 was 6-8 % lower than groups 1 and 2.

The lack of statistical significance above is possibly due to the small sample size.

4.2 Analysis of Serum Lipids (Secondary Study)

4.2.1 Participant Characteristics (n=80)

Vascular biomarkers including biochemical markers were available in a subgroup of 80 participants whose analyses were performed separately in a secondary study. The participant-characteristics of this study are presented in **Table 2.1**, and are similar to those of the main study. The mean±SD age, BMI and WHR of the participants were 60.3±6.3 years, 25.7±3.8 kg/m^2 and, 0.88±0.06, respectively.

Although none of the participants were current smokers, there was approximately equal distribution between the former smokers (51%) and never-smoker (49%) ($p>0.05$).Their mean±SD dCa and dvitD

intake were 858±336 mg/d and 228 (159, 297) IU/d, respectively. The mean±SD values of pSBP, pDBP, and MAP were 114±11, 72±7 and, 83±9 mmHg, respectively.

4.2.2 General Characteristics of Participants in Dietary Calcium Intake Groups

Participant characteristics were examined across the dCa intake groups: 1) <600 mg/d, 2) 600-1000 mg/d, and 3) >1000 mg/d. Results are presented in **Table 2.2.1**. The mean±SD dCa in groups 1, 2, and 3 were 452±94, 810±121, and 1272±217 mg/d, respectively. There were no significant differences in age and BMI among the 3 groups. However, the number of former smokers was significantly lower in group 3 compared to groups 1 (p=0.04) and 2 (p=0.03), respectively. The number of heavy alcohol drinkers (>9 drinks/week) was significantly (p=0.03) higher in group 1 compared to group 2, without any other intergroup significant difference for heavy drinking. Although there was no significant difference of pDBP among the 3 dCa groups, the pSBP (p=0.03) and MAP (p=0.04) were significantly higher in group 3 compared to group 2. There was no significant difference in serum levels of hsCRP and 25(OH)D among the 3 groups. The mean 25(OH)D levels were found to be within normal range across the 3 groups. Moreover, there was no significant difference in the values of serum lipid fractions among groups 1, 2, and 3 in the unadjusted analysis, as presented in **Table 2.2.2**.

4.2.3 Serum Lipids across Dietary Calcium Intake Groups

The serum lipids were compared across the dCa intake groups (1. <600 mg/d, 2. 600-1000 mg/d, and 3. >1000 mg/d) after adjustment for the following covariates: age, BMI, history of smoking, and hsCRP. Results are presented in **Table 2.3**.

Total cholesterol. A non-significant U-shaped relationship between dCa and total cholesterol was observed: adjusted mean value of total cholesterol was lower in group 2 than groups 1 and 3. by 0.11-0.22 mmol/L.

LDL-C. A non-significant U-shaped relationship between dCa and LDL-C was observed, with group 2 having an adjusted mean value 0.03-0.16 mmol/L lower than that of groups 1 and 3.

HDL-C. HDL-C was non-significantly higher in group 1 compared to groups 2 and 3: adjusted mean value of HDL-C in group 2 was 0.07-0.16 mmol/L higher than that of groups 1 and 3.

TAG. TAG levels in group 1 were non-significantly lower compared to those of groups 2 and 3: adjusted mean value of TAG in group 2 was 0.09-0.15 mmol/L lower than those of groups 1 and 3.

ApoB. Adjusted mean value of Apo-B was 0.03 mmol/L non-significantly lower in group 1 compared to groups 2 and 3.

4.3 Main Study: Exploratory Analyses

In our main analysis, we observed that those with dCa 600-1000 mg/d had more favourable values of vascular markers than the rest of our population, although these differences were statistically not significant, possibly due to the small sample size. In order to further characterize this group (600-1000 mg/d), we performed exploratory analyses of vascular markers in this group by further subdividing this group into increments of 200 mg/d dCa, i.e. we compared the vascular parameters between those with 600-800 mg/d, and 800-1000 mg/d of dCa. Results are presented in **Table 3**.

4.4 Subgroup Analyses

In our exploratory analysis (Table 3), we noted that compared to the 600-800 mg/d dCa group, those with 800-1000 mg/d dCa intake had more favourable values of CV markers, particularly for cIMT (0.63±0.08 vs. 0.64±0.09 mm, respectively), and cfPWV (7.0±1.0 vs. 7.8±1.8 m/s respectively), although not statistically significant. Of note, cIMT and cfPWV were our primary vascular markers of interest. Based on these findings, we conducted a subgroup analysis of the main study with 800-1000 mg/d dCa as the reference group and, <800 mg/d and >1000 mg/d as the 2 comparing groups.

4.4.1 Participant Characteristics Across Dietary Calcium Groups

Participant characteristics across dCa groups A (<800 mg/d), B (800-1000 mg/d), and C (>1000 mg/d) are presented in **Table 4.1.1**. The mean±SD values of dCa intake in groups A, B and C were 554±131, 890±56, and 1300±241 mg/d, respectively. The mean age of the participants was only different in group A and C, with group C being significantly older than group A (p=0.03). There was no significant difference in BMI among the 3 groups. However, the number of former smokers was only significantly lower in group C compared to group A (p=0.02). Moreover, the mean intake of dvitD gradually increased from group A to C, where the mean values of dvitD intake was significantly higher in group C compared to groups A (p<0.001) and B (p=0.001), and in group B than group A (p=0.008). In unadjusted analyses, there was no significant difference in the vascular parameters (cIMT, cfPWV, and hemodynamic parameters) among the 3 groups of dCa **(Table 4.1.2)**.

4.4.2 Vascular Parameters across Dietary Calcium Groups

The vascular parameters, namely cIMT, cfPWV, and hemodynamic parameters, were compared across the groups A: <800 mg/d, B: 800-1000 mg/d, and C: >1000 mg/d of dCa after adjustments for relevant covariates (age, BMI, smoking history, MAP, and dvitD intake). Results are presented in **Table 4.2**.

cIMT. Although, there was no statistically significant difference in cIMT among the 3 groups of dCa, cIMT was slightly higher in group C compared to groups A and B. Overall, the adjusted mean value of cIMT in group C was 0.02-0.03 mm higher than groups A and B.

cfPWV. cfPWV in Group B was the lowest among the 3 groups, although this difference was non-significant. The adjusted mean value of cfPWV in group B was 0.4-0.6 m/s lower than that of groups A and C; the highest difference (0.6 m/s) in cfPWV was between group B and group C.

crPWV. crPWV was slightly higher in group C compared to groups A and B; however, this difference was non-significant. The adjusted mean value of crPWV was 0.3-0.4 m/s higher in group C compared to groups A and B.

Peripheral hemodynamic parameters. pSBP and pDBP were slightly higher in group C compared to groups A and B; however, this was non-significant. The adjusted mean values of pSBP and pDBP were numerically 3-4 mmHg, and 1-2 mmHg, higher in group C compared to groups A and B, respectively.

Central hemodynamic parameters. The central hemodynamic parameters, including cSBP, cPP, and MAP, were slightly higher in group C compared to groups A and B; however, there was no significant difference among the groups. The adjusted mean values of cSBP, cPP, and MAP were 1-2 mmHg, 2-4 mmHg, and 4 mmHg higher in group C compared to groups A and B, respectively.

Although there was no significant difference among the 3 groups, a non-significant U-shaped relationship was observed with cDBP in group B as compared to groups A and C.

No difference for the average values of PPA and AP among the groups of dCa was observed.

The mean value of AIx75 was non-significantly higher by 2% in group C than groups A and B.

SEVR in group B was highest among the 3 groups, suggesting a reverse-U shaped relationship between SEVR and dCa intake; the higher the SEVR the bett

TABLES

Table 1.1 – Main Study: Participant Characteristics (n=96)

Variables		Value
Age (y)		60.5 (6.4)
BMI (kg/m^2)		25.6 (3.9)
WHR		0.88 (0.06)
IPAQ (Met-hour/week)		41 (24, 80)
Former-smoker/Never-smoker (%)		51/49
dCa (mg/d)		882 (357)
dvitD (IU/d)		228 (161, 298)
Vascular parameters	**cIMT (mm)**	0.63 (0.57, 0.69)
	cfPWV (m/s)	7.3 (6.4, 8.1)
	crPWV (m/s)	8.0 (1.3)
	pSBP (mmHg)	113 (11)
	pDBP (mmHg)	72 (69, 76)
	cSBP (mmHg)	106 (13)
	cDBP (mmHg)	67 (8)
	cPP (mmHg)	39 (9)
	MAP (mmHg)	83 (9)
	PPA	1.15 (1.10, 1.23)
	AP (mmHg)	13 (5)
	AIx75 (%)	27 (22.5, 29)
	SEVR (%)	164 (33)

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; BMI: body mass index; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; dCa: dietary calcium intake; dvitD: dietary vitamin D intake; IPAQ: international physical activity questionnaire; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification; pSBP: peripheral SBP; SEVR: subendocardial viability ratio; WHR: waist-to-hip ratio. Data presented as mean (standard deviation), median (interquartile range) or percentage.

Table 1.2.1 – Main Study: Participant Characteristics across Dietary Calcium Intake Groups

	Dietary calcium (mg/d)			p-value
	Group 1	Group 2	Group 3	
	<600 (n=25)	600-1000 (n=43)	>1000 (n=28)	
dCa (mg/d)	466 (94)	801 (119)	1300 (241)	
Age (y)	58.1 (5.3)	59.7 (5.5)	62.9 (7.4)*	**0.02**
BMI (kg/m^2)	25.9 (4.2)	25.4 (3.7)	25.7 (3.9)	0.85
Former-smoker/Never-smoker (%)	56/44	60.5/39.5	32/68**	0.05
dvitD (IU/d)	123 (97, 197)	214 (161, 271)*	317 (264, 451)**	**<0.001**

Legend. BMI: body mass index; dCa: dietary calcium intake; dvitD: dietary vitamin D intake; MAP: mean arterial blood pressure.
Data presented as mean (standard deviation), median (interquartile range) or percentage. p-values represent p for differences between group means obtained by ANOVA without adjustments. *: Data significantly different from group 1 dCa; **: Data significantly different from groups 1 and 2 dCa

Table 1.2.2 - Main Study: Vascular Parameters across Dietary Calcium Intake Groups (Unadjusted analysis)

	Dietary calcium (mg/d)			p-value
	Group 1	Group 2	Group 3	
	<600 (n=25)	600-1000 (n=43)	>1000 (n=28)	
cIMT (mm)	0.63 (0.10)	0.63 (0.08)	0.66 (0.11)	0.29
cfPWV (m/s)	7.4 (1.76)	7.4 (1.41)	8.0 (1.45)	0.17
crPWV (m/s)	7.9 (1.55)	7.8 (1.20)	8.2 (1.12)	0.32
pSBP (mmHg)	115 (10)	111 (12)	115 (12)	0.23
pDBP (mmHg)	73 (7)	71 (7)	73 (6)	0.37
cSBP (mmHg)	106 (12)	104 (14)	109 (13)	0.31
cDBP (mmHg)	68 (7)	66 (8)	68 (7)	0.44
cPP (mmHg)	39 (8)	38 (9)	41 (10)	0.46
MAP (mmHg)	83 (8)	82 (10)	86 (9)	0.21
PPA	1.16 (0.10)	1.19 (0.11)	1.18 (0.10)	0.44
AP (mmHg)	14 (5)	13 (6)	14 (6)	0.57
AIx75 (%)	28 (6)	24 (8)	27 (6)	0.11
SEVR (%)	167 (35)	168 (32)	155 (33)	0.22

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification; pSBP: peripheral SBP; SEVR: subendocardial viability ratio. Data presented as mean (standard deviation). p-values represent p for differences between group-means obtained by ANOVA without adjustments.

Table 1.3 - Main Study: Adjusted Means (95% confidence interval) of Vascular Parameters, including cIMT, Arterial Stiffness, and Hemodynamic Parameters across Dietary Calcium Intake Groups

	Dietary calcium (mg/d)			p-value
	Group 1	Group 2	Group 3	
	<600 (n=25)	600-1000 (n=43)	>1000 (n=28)	
cIMT (mm)[¶]	0.63 (0.59, 0.66)	0.63 (0.61, 0.66)	0.67 (0.63, 0.70)	0.35
cfPWV (m/s)[¶]	7.4 (6.8, 8.1)	7.4 (6.9, 7.8)	7.9 (7.2, 8.6)	0.47
crPWV (m/s)[¶]	7.8 (7.3, 8.4)	7.8 (7.5, 8.2)	8.2 (7.6, 8.8)	0.63
pSBP (mmHg)[#]	114 (109, 119)	112 (108, 115)	115 (111, 120)	0.34
pDBP (mmHg)[#]	72 (69, 75)	71 (69, 73)	73 (70, 76)	0.41
cSBP (mmHg)[#]	106 (100, 111)	104 (100, 108)	109 (103, 115)	0.41
cDBP (mmHg)[#]	67 (64, 71)	66 (64, 69)	68 (65, 72)	0.56
cPP (mmHg)[#]	38 (34, 42)	38 (35, 41)	41 (37, 45)	0.54
MAP (mmHg)[#]	83 (79, 88)	82 (79, 85)	85 (82, 90)	0.27
PPA[#]	1.16 (1.11, 1.20)	1.19 (1.16, 1.22)	1.17 (1.13, 1.22)	0.47
AP (mmHg)[#]	14 (11, 16)	13 (11, 15)	14 (12, 16)	0.62
AIx75 (%)[#]	28 (24, 31)	24 (22, 26)	27 (24, 30)	0.11
SEVR (%)[¶]	165 (152, 178)	167 (158, 176)	159 (146, 173)	0.68

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification; pSBP: peripheral SBP; SEVR: subendocardial viability ratio. p-values represent p for differences between group means obtained by ANCOVA after adjustment for covariates (¶: adjusted for age, body mass index [BMI], smoking, vitamin D intake, and MAP; #: adjusted for age, BMI, smoking, and vitamin D intake).

Table 2.1 – Secondary Study: Analysis of Serum Lipids: Participant Characteristics (n=80)

Variables	Value
Age (y)	60.3 (6.3)
BMI (kg/m^2)	25.7 (3.8)
WHR	0.88 (0.06)
IPAQ (Met-hour/week)	41 (25, 83)
Former-smoker/Never-smoker (%)	51/49
dCa (mg/d)	858 (336)
dCa (IU/d)	228 (159, 297)
pSBP (mmHg)	114 (11)
pDBP (mmHg)	72 (7)
MAP (mmHg)	83 (9)

Legend. BMI: body mass index; IPAQ: international physical activity questionnaire, WHR: waist-to-hip ratio; MAP: mean arterial blood pressure; pDBP: peripheral diastolic blood pressure; pSBP: peripheral systolic blood pressure. Data presented as mean (standard deviation), median (interquartile range) or percentage.

Table 2.2.1 - Secondary Study: Participant Characteristics across Dietary Calcium Intake Groups

	Dietary calcium (mg/d)			p-value
	Group 1	**Group 2**	**Group 3**	
	<600 (n=19)	**600-1000 (n=38)**	**>1000 (n=23)**	
dCa (mean±SD, mg/d)	452 (94)	810 (121)	1272 (217)	
Age (y)	59.1 (5.7)	59.6 (5.7)	62.6 (7.5)	0.12
BMI (kg/m^2)	2619 (4.5)	25.5 (3.5)	25.8 (3.7)	0.82
Former-smoker/ Never-smoker (%)	63/37	58/42	30/70**	0.05
Alcohol Heavy drinkers (%)	46[#]	23	31	0.10
pSBP (mmHg)	116 (9)	111 (12)	118 (9)[#]	**0.03**
pDBP (mmHg)	73 (7)	71 (8)	74 (5)	0.26
MAP (mmHg)	83 (8)	81 (10)	87 (8)[#]	**0.04**
hsCRP (mg/L)	1.1 (0.5, 3.1)	0.8 (0.6, 2.3)	1.6 (1.1, 3.9)	0.58
25 (OH)D (nmol/L)	64.0 (55.1, 72.9)	65.6 (59.3, 71.8)	69.0 (60.6, 77.3)	0.63

Legend. BMI: body mass index; dCa: dietary calcium intake; 25 (OH)D: serum 25-hydroxyvitamin D; hsCRP: high-sensitivity C-reactive protein. Data presented as mean (standard deviation), median (interquartile range) or percentage. p-values represent p for differences between group-means obtained by ANOVA without adjustment. **: Data significantly different from groups 1 and 2 dCa; [#]: Data significantly different from group 2 dCa

Table 2.2.2 - Secondary Study: Serum Lipid Values across Dietary Calcium Intake Groups (Unadjusted Analysis)

	Dietary calcium (mg/d)			p-value
	Group 1	**Group 2**	**Group 3**	
	<600 (n=19)	**600-1000 (n=38)**	**>1000 (n=23)**	
Total cholesterol (mmol/L)	5.82 (0.87)	5.66 (0.95)	6.01 (0.91)	0.37
LDL-C (mmol/L)	3.46 (0.81)	3.38 (0.86)	3.67 (0.82)	0.43
HDL-C (mmol/L)	1.91 (0.36)	1.78 (0.31)	1.84 (0.36)	0.41
TAG (mmol/L)	1.01 (0.47)	1.10 (0.55)	1.10 (0.47)	0.82
Apo-B (g/L)	1.03 (0.19)	1.03 (0.25)	1.07 (0.22)	0.80
Apo-A1 (g/L)	1.72 (0.26)	1.60 (0.24)	1.71 (0.21)	0.12

Legend. Apo-B: apolipoprotein-B; Apo-A1: apolipoprotein-A1; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; NS: non-significant; TAG: triglycerides. Data presented as mean (standard deviation). p-values represent p for differences between group means obtained by ANOVA without adjustment

Table 2.3 - Secondary Study: Adjusted Mean (95% Confidence Interval) of Serum Lipids across Dietary Calcium Intake Groups

	Dietary calcium (mg/d)			p-value
	Group 1	Group 2	Group 3	
	<600 (n=19)	600-1000 (n=38)	>1000 (n=23)	
Total cholesterol (mmol/L)$^{\phi}$	5.82 (5.40, 6.24)	5.71 (5.41, 6.00)	5.93 (5.53, 6.32)	0.67
LDL-C (mmol/L)$^{\phi}$	3.46 (3.09, 3.83)	3.43 (3.17, 3.69)	3.59 (3.25, 3.94)	0.77
HDL-C (mmol/L)$^{\phi}$	1.92 (1.78, 2.06)	1.76 (1.66, 1.86)	1.85 (1.72, 1.98)	0.16
TAG (mmol/L)$^{\phi}$	0.98 (0.78, 1.27)	1.13 (0.99, 1.27)	1.07 (0.89, 1.26)	0.44
Apo-B (g/L)$^{\phi}$	1.02 (0.93, 1.12)	1.05 (0.98, 1.12)	1.05 (0.96, 1.14)	0.89
Apo-A1 (g/L)$^{\phi}$	1.74 (1.63, 1.84)	1.60 (1.53, 1.67)	1.71 (1.62, 1.80)	0.05

Legend. Apo-B: apolipoprotein-B; Apo-A1: apolipoprotein-A1; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; NS: non-significant; TAG: triglycerides. p-values represent p for differences between group-means obtained by ANCOVA after adjustment for covariates (ϕ: adjusted for age, BMI, smoking and hs-CRP).

Table 3 - Exploratory Analysis of Main Study: Vascular Parameters including cIMT, and Arterial Stiffness and Hemodynamics across Dietary Calcium Intake Groups (n=43)

	Dietary calcium (mg/d)		p-value
	600-800 (n=18)	**800-1000 (n=25)**	
cIMT (mm)	0.64 (0.09)	0.63 (0.08)	0.72
cfPWV (m/s)	7.8 (1.8)	7.0 (1.0)	0.09
crPWV (m/s)	7.8 (1.2)	7.8 (1.2)	0.99
pSBP (mmHg)	110 (10)	112 (13)	0.72
pDBP (mmHg)	71 (7)	71 (8)	0.99
cSBP (mmHg)	103 (13)	104 (15)	0.86
cDBP (mmHg)	66 (8)	66 (8)	0.91
cPP (mmHg)	37 (9)	38 (10)	0.72
MAP (mmHg)	82 (9)	86 (10)	0.91
PPA	1.20 (0.12)	1.18 (0.10)	0.61
AP (mmHg)	12 (5)	13 (5)	0.61
AIx75 (%)	24 (9)	24 (7)	0.90
SEVR (%)	164 (32)	171 (32)	0.46

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification ratio; pSBP: peripheral SBP; SEVR: subendocardial viability ratio. Data presented as mean (standard deviation). p-values represent p for differences between group-means obtained by independent t-test.

Table 4.1.1- Subgroup Analysis: Participant Characteristics across Dietary Calcium Groups (n=96)

	Dietary calcium (mg/d)			p-value between groups
	Group A <800 (n=43)	Group B 800-1000 (n=25)	Group C >1000 (n=28)	
dCa (mg/d)	554 (131)	890 (56)	1300 (241)	
Age (y)	59.1 (5.5)	59.2 (5.5)	62.9 (7.4)*	**0.03**
BMI (kg/m^2)	25.9 (4.1)	24.9 (3.5)	25.7 (3.9)	0.59
Former-smoker/ Never-smoker (%)	60.5/39.5	56/44	32/68*	0.05
dvitD (IU/d)	159 (111, 203)	253 (179, 279)*	317 (264, 451)**	**<0.001**

Legend. BMI: body mass index; dCa: dietary calcium intake; dvitD: dietary vitamin D intake; MAP: mean arterial blood pressure. Data presented as mean (standard deviation), median (interquartile range) or percentage. *: Data significantly different from <800 mg/d dietary calcium intake group; **: Data significantly different from <800 mg/d and 800-1000 mg/d dietary calcium intake groups.

Table 4.1.2 - Subgroup Analysis: Vascular Parameters Including cIMT, and Arterial Stiffness and Hemodynamics across Dietary Calcium Group (Unadjusted Analysis)

	Dietary calcium (mg/d)			p-value between groups
	Group A <800 (n=43)	Group B 800-1000 (n=25)	Group C >1000 (n=28)	
cIMT (mm)	0.64 (0.09)	0.63 (0.08)	0.66 (0.11)	0.28
cfPWV (m/s)	7.5 (1.76)	7.0 (0.96)	8.0 (1.45)	0.06
crPWV (m/s)	7.8 (1.41)	7.8 (1.22)	8.2 (1.12)	0.34
pSBP (mmHg)	113 (10)	112 (13)	115 (12)	0.47
pDBP (mmHg)	72 (7)	71 (8)	73 (6)	0.55
cSBP (mmHg)	105 (12)	104 (15)	109 (13)	0.38
cDBP (mmHg)	67 (7)	66 (8)	68 (7)	0.52
cPP (mmHg)	38 (8)	38 (10)	41 (10)	0.49
MAP (mmHg)	83 (9)	82 (10)	86 (9)	0.28
PPA	1.17 (0.10)	1.18 (0.10)	1.18 (0.10)	0.96
AP (mmHg)	13 (5)	13 (6)	14 (6)	0.86
AIx75 (%)	26 (8)	24 (7)	27 (7)	0.45
SEVR (%)	166 (33)	171 (32)	155 (33)	0.18

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification ratio; pSBP: peripheral SBP; SEVR: subendocardial viability ratio.

Table 4.2 - Subgroup Analysis: Adjusted Mean (95% Confidence Interval) of Vascular Parameters, Including cIMT, and Arterial Stiffness and Hemodynamic across Dietary Calcium Groups

	Dietary calcium (mg/d)			
	Group A	**Group B**	**Group C**	**p-value**
	<800 (n=43)	**800-1000 (n=25)**	**>1000 (n=28)**	**between groups**
cIMT (mm)[¶]	0.63 (0.60, 0.65)	0.64 (0.61, 0.67)	0.67 (0.63, 0.70)	0.33
cfPWV (m/s)[¶]	7.6 (7.1, 8.1)	7.2 (6.6, 7.7)	7.8 (7.2, 8.5)	0.26
crPWV (m/s) [¶]	7.9 (7.4, 8.3)	7.8 (7.3, 8.3)	8.2 (7.6, 8.8)	0.63
pSBP (mmHg)[#]	112 (108, 115)	113 (109, 117)	116 (111, 121)	0.47
pDBP (mmHg)[#]	72 (69, 74)	71 (69, 74)	73 (70, 76)	0.54
cSBP (mmHg)[#]	104 (100, 108)	105 (100, 110)	109 (104, 115)	0.41
cDBP (mmHg)[#]	67 (64, 69)	63 (66, 69)	68 (65, 72)	0.64
cPP (mmHg)[#]	37 (34, 40)	39 (35, 42)	41 (37, 45)	0.43
MAP (mmHg)[#]	82 (79, 86)	82 (79, 86)	86 (82, 90)	0.33
PPA[#]	1.18 (1.14, 1.21)	1.18 (1.14, 1.22)	1.17 (1.12, 1.21)	0.92
AP (mmHg)[#]	13 (11, 15)	14 (11, 16)	14 (12, 17)	0.71
AIx75 (%)[#]	25 (23, 28)	25 (22, 27)	27 (24, 30)	0.55
SEVR (%)[¶]	165 (155, 175)	168 (156, 179)	159 (146, 173)	0.66

Legend. AIx75: AIx corrected for a heart rate of 75 bpm; AP: augmentation pressure; cDBP: central diastolic blood pressure; cfPWV: carotid-to-femoral pulse wave velocity; cIMT: carotid intima-media thickness; crPWV: carotid-to-radial PWV; cSBP: central systolic blood pressure; MAP: mean arterial blood pressure; pDBP: peripheral DBP; PPA: pulse pressure amplification; pSBP: peripheral SBP; SEVR: subendocardial viability ratio. ¶: adjusted for age, body mass index (BMI), smoking, vitamin D intake, and MAP; #: adjusted for age, BMI, smoking, and vitamin D intake.

5.0 DISCUSSION

In this cross-sectional study of 96 healthy postmenopausal women, dCa was not significantly associated with CV markers, including cIMT, cfPWV, and hemodynamic parameters (Main Study). Moreover, analysis in a subgroup of 80 participants, there was no significant association between dCa and serum lipids (Secondary Study).

Although statistically non-significant, we noted that there may be favorable values of CV markers in those with <1000 mg/d vs. >1000 mg/d as well those in the middle groups of dCa (600-1000 mg/d or 800-1000 mg/d) compared to the extreme groups (<600 mg/d, <800 mg/d, or >1000 mg/d). The lack of significance could be attributed to the small sample size of the study group. These differences suggest that optimum level of calcium intake as compared to extremes of (low/high) dCa may be associated with better CV health parameters. Importantly, our entire study population had optimal/normal CV markers values with respect to their age.

5.1 Main study and Subgroup Analysis: Analysis of Vascular Parameters

Canadian guidelines recommend 3 portions of dCa every day for women over 50 years of age, which is similar to the age group of our study population. Of note, each portion is equivalent to approximately 300 mg of elementary calcium.[282] Hence, we selected our reference group for the main study as those with 600-1000 mg/d dCa, which is equivalent to 2-3 portions dCa/d, and <600 mg/d and >1000 mg/d dCa as the other comparison groups. Furthermore, we observed in the exploratory analysis of main study that those with 800-1000 mg/d dCa had lower values for cIMT and cfPWV, our primary vascular markers of interest. Hence, we performed a subgroup analysis with the participants having 800-1000 mg/d dCa as reference group, and <800 mg/d and >1000 mg/d dCa groups as the other comparison groups.

Although statistically non-significant, the average value of cIMT in those with >1000 mg/d was higher compared to the group with <1000 mg/d dCa. In a RCT in dyslipidemic participants, a significant interaction between calcium supplementation and menopausal status on cIMT (p=0.017) was reported, and those postmenopausal women who took 800 mg/d of elemental calcium supplementation for 2 years had significantly higher increase in cIMT than premenopausal women (0.06±0.13 mm vs.0.02±0.03 mm, respectively, p=0.003).[220] Reid et al. proposed that calcium supplements may predispose vascular calcification as a result of the complexing of calcium ions with inhibitors of calcification, or may cause endothelial dysfunction, or may enhance atherosclerosis through calcium-induced altered PTH metabolism, all of which may have an adverse effect on vascular health.[97] Li et al. also proposed that calcium supplements might affect cholesterol metabolism in a menopausal estrogen-deficiency state thereby predisposing to increased cIMT and carotid atherosclerosis in postmenopausal women only .[220,229] However, another RCT in postmenopausal women (mean age=75.2±2.7 years), which randomized women to either 1.2 g of elemental calcium supplementation or placebo for 3 years, reported no difference in cIMT (mean cIMT=0.778±0.006 mm vs. 0.783±0.006 mm, respectively, p=0.491) between the 2 groups. Moreover, the authors also reported a significant reduction in carotid atherosclerosis in those with at least 80% compliance with calcium supplements (54.7 % in the supplement group vs. 46.7 % in the placebo group, p=0.033), as well as in those in the highest tertile (>1795 mg/d) vs. the lowest tertile (<1010 mg/d) of total calcium intake (OR of participants with carotid atherosclerosis=0.70; 95% CI: 0.51–0.96).[284]

When compared to interventional studies, observational studies did not find any significant association between calcium intake and cIMT. A prospective cohort study in 1026 middle-aged participants (mean age in men=52.5±4.7 years, and in women=52.3±4.6 years) followed up for

7.5 years reported no definite association between a dietary pattern characterized by low intakes of calcium and cIMT (multivariable adjusted cIMT for 1st tertile=0.702±0.004 mm, 2nd tertile=0.705±0.004 mm, and 3rd tertile=0.696±0.004 mm; p=0.36).[285] Similarly, another prospective cohort study involving obese subjects (n=44; mean age=67. 4±5.3 years; men=61.4%) with low habitual dCa (556±266 mg/d) showed no association between dCa and cIMT.[286] Moreover, a cross-sectional analysis in 592 relatively healthy men and women (mean age=48.2 years) reported no association between total calcium intake and cIMT following adjustment for age and sex (calcium intake as a predictor of cIMT: intercept=0.00001347; 95% CI: 0.00003039-0.00000345; p=0.1184).[287]

We noted that cIMT in those with >1000 mg/d dCa was 0.02-0.04 mm thicker than in those with <1000 mg/d dCa. This finding is relevant as an increase in cIMT has been shown to be a predictor of CVD and CV events: an increase in cIMT of 0.20 mm in the CHS (n=4476 healthy subjects; mean age=72.5±5.5 years) was associated with a 27% increased risk (RR: 1.27; 95% CI: 1.17–1.38) for stroke or MI with a follow-up period of 6.2 years.[181] Similarly, the CAPS (n=5056; mean age=50.1 years) observed adjusted HR of 1.16 (95% CI: 1.05–1.27) for MI, and 1.17 (95% CI: 1.08–1.26) for the combined endpoint of MI, stroke or death per 0.16 mm increase in cIMT when the participants were followed up for 4.2 years.[186] Likewise, the ARIC study (n=7289 women and 5552 men without CHD at baseline, age range=45-64 years, follow-up duration=4-7 years) reported a HR of 1.38 (95% CI: 1.21-1.58) for women, and 1.17 (95% CI: 1.04-1.31) for men for the incidence of CHD for every 0.19 mm increase in cIMT.[207]

A non-significant dose-response relationship was also noted in our study between dCa and PWV. cfPWV in the main analysis, and crPWV in the main as well as the subgroup analysis in those with >1000 mg/d dCa were non-significantly higher than dCa groups with intake <1000 mg/d.

However, in the subgroup analysis, the value of cfPWV was non-significantly lower in 800-1000 mg/d dCa than the extreme groups (<800 mg/d and >1000 mg/d), suggesting a possible U-shaped dose response association between dCa and cfPWV.

The association between calcium intake and PWV has been examined in few studies. Although statistically non-significant, an interventional study in healthy subjects (mean age=60.3±6.5 y) observed higher cfPWV (8.9 m/s vs. 8.2 m/s, p=0.33) 3 hours following a single oral dose of 1000 mg calcium citrate when compared to baseline.[101] A small RCT in healthy postmenopausal women studying the effect of 1200 mg of calcium either from diet (n=4) or from supplements (n=5) for 12 months also did not report any significant between-group or within-group changes in cfPWV or crPWV. However, while statistically not significant, within-group analysis showed there was an increase in mean cfPWV of 0.45 m/s in the supplemental group vs. a decrease of 1.41 m/s in the dietary group over 12 months, suggesting that the source of calcium intake, dietary or supplemental, may have a differential impact on vascular health.[288]

On the other hand, a cross-over RCT in healthy volunteers (mean age=33±6.1 years) at 2 hours following an intervention of 600 mg of calcium either dietary or supplemental, reported a fall in cfPWV irrespective of the source of calcium (supplemental group=8.85±1.0 m/s vs. 8.72±1.3 m/s, and dietary intake group=8.54±1.2 m/s vs. 8.21±1.5 m/s). However, the results were not statistically significant (both, p>0.05).[164] Similarly, Crichton et al.,[165] in a cross-sectional analysis of healthy subjects (mean age=63-65.2 years), reported a linear decrease in cfPWV (never/seldom dairy intake=11.0 m/s, 1 time/week=10.8 m/s, 2-4 times/week=10.6 m/s, 5-6 times/week=10.0 m/s, ≥1/d=10.1m/s; p-trend=0.018) with increasing intakes of dairy food consumption, the principal source of dCa. Likewise, in a prospective cohort study in 1026 participants (mean age in men=52.5±4.7 years and women=52.3±4.6 years) followed up for 7.5

years reported a positive association between a dietary pattern characterized by low intake of calcium and cfPWV (adjusted mean cfPWV across tertiles [T]: T1=11.15 m/s, T2=11.26 m/s and T3=11.58 m/s, respectively; p-trend=0.03), suggesting calcium intake may have an inverse relationship with arterial stiffness.[285] Furthermore, in a cross-sectional study in Japanese men, aged 35-69 years, a significant inverse association between brachial-ankle PWV (baPWV, another marker of arterial stiffness[135]) and increased dCa (multivariable adjusted mean±standard error baPWV [m/s] in quartiles [Q] of dCa: Q1 [≤351.8 mg/d]=15.61±0.29, Q2 [>351.8–412.2 mg/d]=15.07±0.27, Q3 [>412.2–497.3 mg/d]=15.12±0.28, Q4 [>497.3 mg/d]=15.05±0.28; p-trend=0.020) was observed.[166]

In our study, we demonstrated a non-significant 0.5 m/s higher cfPWV in those with >1000 mg/d dCa than those with <1000 mg/d intake in the main analysis, and 0.4-0.6 m/s higher cfPWV in those with <800 and >1000 mg/d vs. 800-1000 mg/d dCa in the subgroup analysis. This is important as cfPWV has a high predictive value for CVD, as reported by a meta-analysis showing that an increase in cfPWV by 1 m/s corresponded to an age-, sex-, and risk factor-adjusted risk increase of 14%, 15%, and 15% in total CV events, CV mortality, and all-cause mortality, respectively.[138]

In our study, pBPs had a non-significant U-shaped relationship with dCa in the main analysis, where pSBP and pDBP in the 600-1000 mg/d dCa group were lower compared to the extreme groups (<600 mg/d and >1000 mg/d dCa). In the subgroup analysis, peripheral BPs in the >1000 mg/d dCa group were non-significantly higher compared to the group with <1000 mg/d dCa. In both main study and subgroup analyses, cSBP was non-significantly higher in the >1000 mg/d vs. <1000 mg/d dCa groups, while cDBP was non-significantly lower in the middle groups of dCa (600-1000 mg/d for main analysis, and 800-1000 mg/d for subgroup analysis) compared to

the extreme groups (<600 mg/d and >1000 mg/d for main analysis, and <800mg/d and >1000 mg/d for subgroup analysis, respectively). Overall, both pBPs and cBPs were non-significantly lower, either for those with <1000 mg/d dCa compared to the rest of the study population, or for those in the middle groups of dCa (600-1000 mg/d or 800-1000 mg/d) vs. the extreme groups (<600 mg/d, <800 mg/d and >1000 mg/d). However, BPs in all 3 groups of dCa in both the main study and the subgroup analysis were within normal limit for their age (<120/<80 mmHg).

The Korea National Health and Nutrition Examination Survey (KNHANES 2008-2011) reported a U-shaped relationship between dCa and pSBP (<600 mg/d: 119-122 mmHg, 600-900 mg/d: 115 mmHg, and >900 mg/d: 118-119 mmHg; p<0.001), as well as pDBP (<600 mg/d: 76 mmHg, 600-900 mg/d: 74 mmHg, and >900 mg/d: 75-76 mmHg; p=0.009) in Korean women.[289] An interventional study comparing the effect of calcium intake (1200 mg/d) either from diet or supplements reported an increase in pSBP (baseline vs. after 12 months: 114 mmHg vs. 125 mmHg) and pDBP (67 mmHg vs. 76 mmHg) after 12 months of intervention with calcium supplements, while there was no appreciable change in BPs (baseline vs. 12 months pSBP: 104 vs. 104 mmHg, baseline vs. 12 months pDBP: 71 vs. 70 mmHg) following dietary intervention, However, none of the changes were statistically significant.[288] Therefore, although our results are non-significant, they reflect the overall trend reported by other studies.

Most studies either reported a neutral effect or reduction on BP with increasing calcium intake. Subgroup analysis of a meta-analysis of epidemiological studies reported an inverse association between dCa and BP: a significant reduction of pSBP and pDBP of 0.010 mmHg ($p < 0.001$) and 0.009 mmHg ($p < 0.05$), respectively, in men, and 0.15 mmHg ($p < 0.001$) and 0.057 mmHg ($p < 0.02$), respectively, in women was reported for every 100-mg increase in dCa.[245] Similarly, another meta-analysis of RCTs reported that calcium supplementation (1200 mg/d) in

comparison to placebo reduced pSBP by 1.86 mmHg (95% CI: -2.91 to -0.81) and pDBP by 0.99 mmHg (95% CI: -1.61 to -0.37).[290] Moreover, a subgroup analysis of this study showed that those with ≤1000 mg/d calcium supplementation had a higher decrease in pSBP (-2.17; 95% CI: -3.59 to -0.75, vs. -1.75; 95% CI: -3.20 to -0.31), and pDBP (-1.41; 95% CI: -2.24 to -0.59, vs. -0.56; 95% CI: -1.40 to 0.29) compared to those with >1000mg/d supplementation, implying <1000 mg/d vs. >1000 mg/d calcium intake may have a beneficial effect on CV health,[290] as hinted by our own non-significant observations. Likewise, another meta-analysis reported a reduction in pSBP by 1.44 mmHg (95% CI: -2.20 to -0.68, $p < 0.001$) and in pDBP by 0.84 mmHg (95% CI: -1.44 to -0.24, $p < 0.001$) with calcium supplementation when compared to placebo.[291]

We reported non-significant and modest 2-4 mmHg and 1-2 mmHg reductions in pSBP and pDBP, respectively, either for those with <1000 mg/d vs. >1000 mg/d dCa groups, or for those in the middle groups as compared to the extreme groups of dCa (600-1000 mg/d vs. <600mg and >1000 mg/d in the main analysis and, 800-1000 mg/d vs. <800 mg/d and >1000 mg/d in the subgroup analysis). This is important, as a few studies reported that a reduction in BPs is translated into a reduction in CV morbidity and mortality. A first meta-analysis of prospective longitudinal studies reported a 21% decreased risk of CHD with a 5 mmHg reduction in pDBP,[292] while another meta-analysis reported 25% decreased risk of CHD with either 5 mmHg or 10 mmHg reduction of pDBP or pSBP, respectively. Moreover, the latter study demonstrated an age-dependent trend in the risk of CHD with every 10 mm reduction in pSBP: RR for those <60 years: 0.55 (95% CI: 0.52–0.58), RR for those 60–69 years: 0.70 (95% CI: 0.66–0.75), RR for those >70 years: 0.82 (95% CI: 0.78–0.86).[293] Furthermore, other meta-analyses[234,294] reported a decreased risk of stroke with reduction in BPs: for every 10 mmHg reduction in pSBP,

there was a 37 % decreased risk of stroke, as well as a trend for a decrease in stroke risk with increasing age for similar reduction of BP values.[294]

We reported 1-5 mmHg lower cSBP in dCa groups with <1000 mg/d vs. those with >1000 mg/d, which might have an impact on CV outcomes, as reported in few studies. The Strong Heart study in 2403 participants free from CVD and followed up for 4.8±1.3 years, reported that increased cSBP was associated with enhanced risk of CV events (HR per 10 mmHg increase: 1.07; 95% CI: 1.01–1.14) similarly to increased pSBP (HR per 10 mmHg increase: 1.08; 95% CI: 1.02–1.14).[295] Moreover, the Insufficienza Cardiaca negli Anziani Residenti a Dicomano (ICARe Dicomano) Study in 398 subjects followed up for about 8 years, reported increased cSBP (HR per 10 mm increase: 1.19; 95% CI: 1.08–1.31; p<0.0001), but not pSBP (p=0.119), to be associated with increased risk of CV events.[296] This was further confirmed by a meta-analysis of longitudinal studies that demonstrated an increased risk of CV events with increased cSBP (RR per 10 mmHg increase of cSBP: 1.088; 95% CI: 1.040–1.139).[297]

In both our main study as well as the subgroup analysis, we reported non-significant higher cPP in those with >1000 mg/d compared to dCa groups with <1000 mg/d intake. Contrary to our results, a cross-sectional analysis in healthy men and women (mean age=63-65.2 years) reported a linear decrease in peripheral PP (pPP) (never/seldom dairy intake=57.6 mm, 1 time/week=53.1 mm, 2-4 times/week=52.4 mm, 5-6 times/week=50.8 mm, ≥1/d=51.6 mm; p-trend=0.013) with increasing intakes of dairy food consumption, the principal source of dietary-calcium. Although non-significant, 2-4 mm higher value of cPP in those with >1000 mg/d vs. <1000 mg/d dCa noted in our study is important, as higher cPP is associated with increased CV risk.[295-298] Importantly cPP has been reported to have better predictive value for CVD than pPP.[295,296,298] A

meta-analysis of prospective studies reported a 14% (RR: 1.137; 95% CI: 1.063 –1.215) increased risk in CV events for every 10 mm increase in cPP.[297]

There was also a non-significant 2-4 mm higher value of MAP in those with >1000 mg/d compared to dCa groups having <1000 mg/d in both the main study and subgroup analysis. Prospective studies reported that a higher MAP was predictive of CVD: the Chicago Heart Association Detection Project in Industry Study (CHADP-IS) in 60-74 years old women noted a 27% increased risk for CVD (HR: 1.27; 95% CI: 1.13-1.44) per SD increase in MAP (equivalent to 13.6 mmHg) which was primarily driven by a 32 % increased risk for CHD (HR: 1.32; 95% CI: 1.14-1.54); interestingly the age range of the CHADP-IS population was similar to the age span of our study population. [299] Similarly, a prospective study in men without any history of CV risk factors at baseline followed up for about 11 years, reported a 28-48 % increased risk of CVD with every 10 mmHg increase of MAP.[300]

A non-significant reverse U-shaped association between calcium intake and PPA was observed in the main study, where PPA in those with 600-1000 mg/d dCa was 0.02-0.03 (2-3 %) higher compared to the extreme groups, i.e. <600 and >1000 mg/d intakes; however, there were no difference in the mean PPA values among the groups of dCa in the subgroup analysis. PPA, an indicator of central arterial stiffness,[140,141] has been reported to have a high predictive value for CV outcomes: the Predictive Values of Blood Pressure and Arterial Stiffness in Instituitionalised Very Aged Population (PARTAGE) study in nursing home dwellers noted a 17% ($p<0.01$) decrease in major CV events, and a 24% ($p<0.01$) decrease in total mortality with every 10% increase in PPA.[301]

In the main study, those with 600-1000 mg/d dCa had lower value of AIx75 in comparison to the extreme groups (<600 mg/d and >1000 mg/d) of dCa, while in the subgroup analysis the dCa

groups with intake <1000 mg/d had lower value of AIx75 than those with >1000 mg/d dCa. An interventional study noted a significant decrease in the median value of AIx (from 29.7% to 26.4%; p <0.05) 3 hours after a single oral dose of 1000 mg calcium citrate.[101] Our study shows a non-significant 2-4 % decrease in AIx75 in both the <1000 mg/d vs. >1000 mg/d groups (main study), or the 800-1000 mg/d vs. the extreme groups (<800 mg/d and >1000 mg/d) (subgroup analysis) of dCa, which is of interest, as a 10 % elevation in AIx has been reported in a meta-analysis to be associated with 32% and 38% increased risk of CV events (RR: 1.32; 95% CI: 1.09–1.59), and all-cause mortality (RR: 1.38; 95% CI: 1.19 –1.61), respectively.[297]

In our main study, SEVR in those with >1000 mg/d was non-significantly 6-8% lower than in those with <1000 mg/d dCa; however, in the subgroup analysis, there was a non-significant reverse-U shaped relationship between dCa and SEVR, where those with 800-1000 mg/d had 3-9% higher SEVR compared to the extreme groups (<800 mg/d and >1000 mg/d groups) of dCa, suggesting that increased calcium intake might lead to a reduction in SEVR, a marker of increased risk for CVD.[152,153] Contrary to our findings, an interventional study using 1000 mg calcium supplementation in healthy subjects resulted into a significant increase of 7% in the value of SEVR 3 hours following intervention.

5.2 Secondary Study: Analysis of biomarkers

In our biomarkers analysis, those with 600-1000 mg/d dCa had 0.11-0.22 mmol/L lower levels of total cholesterol, than the extreme groups of dCa, i.e. <600 mg/d and >1000 mg/d. This is important as a meta-analysis studying the relationship between blood cholesterol and vascular mortality reported that a 1 mmol/L lower total cholesterol was associated with 56% (HR: 0.44; 95% CI: 0.42–0.48), 34% (HR: 0.66; 95% CI: 0.65–0.68), and 17% (HR: 0·83; 95% CI: 0.81–0.85) lower IHD mortality in both sexes in those of ages 40–49, 50–69, and 70–89 years,

respectively.[302] Similarly, the Eastern Stroke and Coronary Heart Disease Collaborative Research Group reported reduced risk of non-hemorrhagic stroke with decreased levels of serum total cholesterol (OR: 0·77; 95% CI: 0.57–1.06 per 0.6 mmol/L decrease in total cholesterol).[303] A non-significant, U-shaped relationship between dCa and total cholesterol, primarily driven by LDL-C, was noted in our study: those with 600-1000 mg/d dCa had lower levels of total cholesterol and LDL-C than the extreme groups, i.e. <600 mg/d and >1000 mg/d dCa. Similar results were reported in the KNHANES (2008-2011), where women (n= 4357, age>40 years) having 600-900 mg/d dCa had lower total cholesterol (5.01 mmol/L) compared to those with <600 mg/d (5.05-5.06 mmol/L) and >900 mg/d (5.01-5.23 mmol/L) dCa.[289] Calcium intake-induced altered cholesterol metabolism was also observed in an interventional study in dyslipidemic women receiving either 800 mg Ca/d or a placebo for 2 years. In this study, total cholesterol levels increased significantly in the calcium supplementation group compared to the placebo group (placebo vs. supplementation: 5.56±0.64 vs. 5.87±0.7; p <0.001); although there was no significant between-group change, a non-significant increase in LDL-C level (placebo vs. supplementation: 3.49±0.69 vs. 3.54±0.67, p=0.712) was observed.[220] Moreover, the same study reported a significant (p<0.001) difference in the change of total cholesterol between premenopausal women (0.04±0.07 mmol/L) vs. postmenopausal women (0.61±0.21 mmol/L) following calcium supplementation.[220] Calcium is thought to inhibit cholesterol catabolism in postmenopausal women as a result of estrogen deficiency following menopause.[229]

However, calcium intake has been reported in other studies to alleviate total cholesterol, as well as LDL-C levels. Calcium interacts with fatty acids and bile acids in the gut to interfere with their intestinal absorption, which leads to increased fecal excretion of lipids.[226] Moreover, calcium intake might also have a beneficial effect on lipids by favourably modulating the gut

environment for lactobacilli,[227] or by its effect on calcitropic hormones promoting lipolysis.[228,304] Such mechanistic explanation was suggested in a study demonstrating lowering of total cholesterol (-0.18±0.72 mmol/L), and LDL-C (-0.27± 0.60 mmol/L) following 1000 mg/d calcium supplementation for 1 year in an RCT in healthy postmenopausal women.[224] Similarly, in a subgroup analysis of participants without history of hypercholesterolemia in another RCT (n=193 men and women), receiving either >1000 mg of elemental calcium supplementation or placebo for 4 months, the decline in total cholesterol was 0.18 mmol/L (p=0.10) more in the calcium supplementation group than in the placebo group.[305] However, in the same study, the HDL-C levels, the protective cholesterol fraction, dropped 0.02 mmol/L (p=0.61) more in the calcium supplementation group than in the placebo group,[305] suggesting that calcium might also be involved in the altered metabolism of HDL-C. Calcium intake-induced altered metabolism of HDL-C was also observed in our study, where increased calcium intake was associated with a fall in HDL-C levels: HDL-C was 0.07-0.16 mmol/L lower in those with >600 mg/d vs. those with <600 mg/d dCa. Li et al. also reported in dyslipidemic women a decline in HDL-C following 800 mg/d calcium supplementation for 2 years (baseline vs. at 2 years: 1.23±0.14 mmol/L vs. 1.20±0.14 mmol/L), although the change was not statistically significant.[220]

Among the different factors affecting HDL-C metabolism is alcohol intake.[306] The higher proportion of heavy drinkers (>9 drinks/week) in the <600 mg/d dCa group (46%) compared with the 600-1000 mg/d (23%) and the >1000 mg/d (31%) groups might explain the slightly elevated HDL-C in the <600 mg/d compared to the >600 mg/d dCa groups in our study. However, the KNHANES (2008-2011) study reported a trend of increasing serum HDL-C with increasing dCa (<300 mg/d: 1.38 mmol/L, 300-600 mg/d: 1.40 mmol/L, 600-900 mg/d: 1.43

mmol/L, 900-1200 mg/d: 1.41 mmol/L, >1200 mg/d: 1.49 mmol/L; p<0.01) in women, although the alcohol consumption in each of the groups were not reported in the study.[289] Similarly, calcium intake-induced elevation in HDL-C was observed in an RCT in healthy postmenopausal women receiving either 1000 mg elemental calcium supplementation or placebo for 12 months: there was a 0.09 mmol/L (95% CI=0.02-0.17, p=0.01) greater increase in the HDL-C in the supplementation group compared to the placebo group at the end of the intervention period. Of note, there was no significant difference (p=0.66) in alcohol consumption between the two groups.[224]

In the same RCT,[224] although there was a decrease in TAG levels both in the supplementation group (-0.02±0.35 mmol/L), as well as placebo group (-0.06±0.46 mmmol/L) after 12 months of calcium supplementation, there was a 0.05 mmol/L (95% CI=-0.08-0.17) lower (p=0.48) decrease in the levels of TAG in the intervention group compared to the placebo group. This suggests calcium might slow down TAG catabolism. Similarly, our study's results also hinted that calcium intake may induce altered TAG metabolism, where we observed that those with >600 mg/d dCa had 0.09-0.15 mmol/L higher TAG compared to those with <600 mg/d intake. Likewise, Kim et al. in a cross-sectional analysis in healthy subjects reported a positive correlation between dCa and TAG (r=0.3665, p < 0.01), primarily driven by the intake of calcium from animal sources, including dairy calcium (r=0.4003, p < 0.001).[307] Similarly, Li et al. reported an non-significant increase in TAG following 800 mg/d calcium supplementation for 2 years in an interventional study of dyslipidemic women (baseline vs. at 2 years following intervention: 1.69±0.63 vs. 1.73±0.63 mmol/L).[220] However, KNHANES (2008-2011) reported a U-shaped relationship between dCa and TAG levels in women (n=4357, age >40 years): those

with 600-900 mg/d dCa had lower TAG levels (1.18 mmol/L), compared to those with <600 mg/d (1.38-1.45 mmol/L), and >900 mg/d (1.24-1.35 mmol/L) intakes.[289]

Apo B is the major apolipoprotein constituent of LDL and non-HDL, and is correlated with the concentrations of these cholesterol fractions.[308] A small 0.03 mmol/L higher level of Apo-B in >600 mg/d than <600 mg/d dCa groups noted in our study could be primarily driven by higher concentration of LDL-C (unadjusted mean: 3.53 vs. 3.46 mmol/L) and non-HDL-C (unadjusted mean: 4.02 vs. 3.90 mmol/L) concentration in >600 mg/d than <600 mg/d dCa groups.

Dose response relationships between calcium intake and CV outcomes were reported in meta-analyses and prospective studies. Wang et al., in a meta-analysis of prospective studies, reported a U-shaped association between dCa and CV mortality, with the lowest risk associated with 800 mg/d dCa consumption compared to < 800 mg/d and >800 mg/d.[76] In another meta-analysis evaluating the association between dCa and stroke, a U-shaped association was observed with the lowest risk of stroke around 1000 mg/d dCa.[77] CaMos noted a 22% reduced risk (HR: 0.78; 95% CI: 0.66-0.92) in all-cause mortality for women users of calcium supplements when compared to supplement non-users. However, this beneficial effect was absent beyond 1000 mg/d (HR: 0.88; 95% CI: 0.65-1.18).[309] Similarly, in men only in the NIH-AARP study, there was an elevated risk for CVD death (RR: 1.20; 95% CI: 1.05–1.36) for those with >1000 mg/d calcium supplement use when compared to supplement non-users.[82] In the same study, a U-shaped relationship between total calcium intake and CVD mortality was noted, with the lowest risk for those with around 1000 mg/d total calcium intake.[82] Likewise, the Swedish mammography cohort study in about 60,000 women followed for 19 years reported a U-shaped dose-response relationship between dietary and total calcium intake and CV, as well as IHD mortality, with the lowest risk in those with 600-1000 mg/d intake.[6] Comparable to the above

mentioned studies, a dose-response relationship between dCa and CV health markers was observed in our study, where we noted favourable levels of CV markers in the <1000 mg/d vs. >1000 mg/d dCa groups, and in the 600-1000 mg/d (main study), or 800-1000 mg/d dCa groups than the extreme groups (U-shaped association).

6.0 Limitations

Our study has several limitations. Firstly, this study is observational, and therefore could not assess the causal relationship between dCa and CV health. Secondly, the cross-sectional nature of the study could not explore the cumulative effect of dCa on CV health over time, as noted in longitudinal prospective studies. Moreover, the assessment of dietary calcium intake by food frequency questionnaire might be limited by recall bias. Although clinically relevant relationship was noted, the small sample size of our study limited the statistical power to detect significant association between dCa and CV markers.

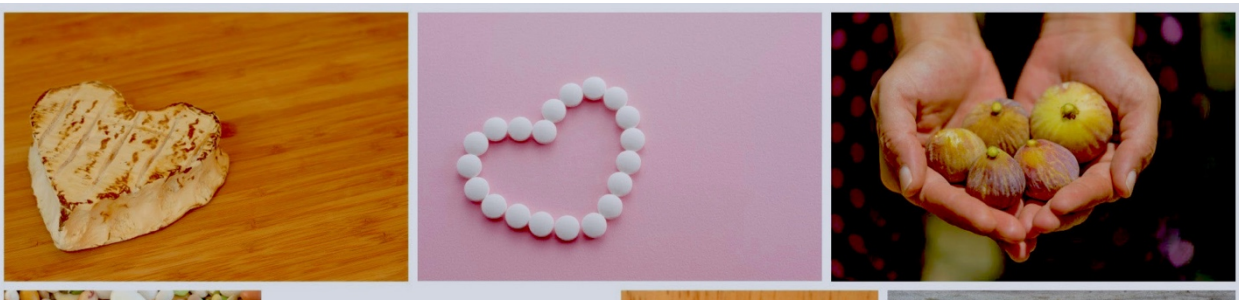
7.0 Conclusions

In this cross-sectional study of healthy postmenopausal women, we investigated the relationship between dCa and CV health markers, i.e., cIMT, arterial stiffness parameters, including cfPWV and other hemodynamic parameters, and serum lipids. Although not statistically significant, favourable values of CV markers were observed for those with <1000 mg/d vs. >1000 mg/d dCa, and for those with 600-1000 mg/d vs. <600 mg/d and >1000 mg/d dCa (U-shaped relationship). The best CV profile in those with 600-1000 mg/d dCa was primarily driven by the most favourable values of CV parameters in those with 800-1000 mg/d compared to the rest of the population. Our results suggest that the middle range of dCa (600-1000 mg/d or 800-1000 mg/d) as opposed to extremes, i.e. low (<600 mg/d or <800 mg/d) or high (>1000 mg/d) dCa, might be associated with better CV makers. Further larger studies are needed to confirm the observed associations between dCa and CV subclinical markers in postmenopausal women as well as to assess whether their longitudinal changes are associated with the development of CVD.

8.0 Future Directions

The present study is a cross-sectional analysis of data collected at baseline in women participating in our ongoing longitudinal CIHR-funded RCT (ClinicalTrials.gov NCT01731340), the Calcium Study, which investigates the effect of dCa vs. supplemental calcium on CV health. Recruitment for the Calcium Study is still underway, with inclusion of more study participants, which will allow for a greater statistical power necessary to detect clinically, as well statistically, significant associations between dCa and CV markers. Hence, the ongoing RCT will allow for better evaluation of the effect of calcium intake on CV health. Moreover, given the parabolic (U-shaped) relationship between calcium intake and vascular parameters, we will explore quadratic relationship between these variables. Furthermore, we will assess the interaction of calcium intake and kidney function on the CV markers. Additionally, we plan to assess novel CV biomarkers, including inflammatory markers, from the appropriately stored fasting blood samples to explore the association of dCa and CV health in a more comprehensive way.

Appendix A: Calcium Study Advertisement



Concerned about calcium?

Préoccupée par le calcium?

FACT: Postmenopausal women need 1200 mg of calcium every day to keep their bones strong.

FACT: Most postmenopausal women rely on supplements to get enough calcium daily.

QUESTION: We know that calcium supplements are good for the bones, but is it possible that they are bad for the heart?

As researchers at the McGill University Health Centre, we are conducting a study on calcium to answer this question. We want to know if calcium supplements have a different effect on vascular health in postmenopausal women compared to calcium obtained from food.

You can help answer these important questions by participating in our study!

ImpactCalcium

Centre universitaire de santé McGill — McGill University Health Centre

CIHR IRSC
Canadian Institutes of Health Research — Instituts de recherche en santé du Canada

ClinicalTrials.gov
NCT01731340

You may qualify to participate if:
- you are a healthy non-smoker over the age of 50
- you have had no menstrual period for at least 2 years
- you are not taking HRT, or medication for high blood pressure, high cholesterol, or osteoporosis

For the period of a year, you must be willing to:
- alter the amount of calcium in your diet
- take or abstain from taking calcium supplements

Participants will receive all supplements at no cost and will be reimbursed for transportation or parking fees.

Principal Investigators: Dr. Suzanne Morin and Dr. Stella Daskalopoulou
Research funded by: Canadian Institutes of Health Research
Location: Montreal General Hospital, McGill University Health Centre
Requirements: 3 visits to complete questionnaires, provide blood and urine samples, and undergo ultrasounds of your arteries

FAIT : Les femmes ménopausées ont besoin de 1200 mg de calcium chaque jour pour garder leurs os solides.

FAIT : La plupart des femmes ménopausées comptent sur des suppléments pour obtenir assez de calcium quotidiennement.

QUESTION : Nous savons que les suppléments de calcium sont bons pour les os, mais se pourrait-il qu'ils soient dangereux pour le cœur?

En tant que chercheuses au Centre universitaire de santé McGill, nous menons une étude sur le calcium pour répondre a cette question. Nous cherchons à savoir si les suppléments de calcium ont un effet différent sur la santé vasculaire chez les femmes ménopausées comparativement au calcium obtenu des aliments.

Vous pouvez aider à répondre à ces questions importantes en participant à notre étude!

Vous pourriez être admissible à l'étude si :
- vous êtes une non-fumeuse en bonne santé âgés de plus de 50 ans
- vous n'avez pas eu vos menstruations depuis au moins 2 ans
- vous ne prenez pas d'hormones, d'antihypertenseurs, ou de médicaments contre le cholestérol ou l'ostéoporose

Pendant une période d'un an, vous devez être prête à :
- modifier la quantité de calcium contenue dans votre alimentation
- prendre ou vous abstenir de prendre des suppléments de calcium

Les participantes recevront les suppléments sans frais et elles seront remboursées pour les frais de déplacement ou de stationnement.

Chercheuses Principales : Dre Suzanne Morin et Dre Stella Daskalopoulou
Recherche financée par : Instituts de recherche en santé du Canada
Lieu : Hôpital général de Montréal, Centre universitaire de Santé McGill
Exigences : 3 visites pour compléter des questionnaires, fournir des échantillons de sang et urine, et passer des échographies de vos artères

For more information | Pour plus d'information

calcium.medicine@mcgill.ca | 514-934-1934 ext. 45742 | http://www.mcgill.ca/morin-lab/calcium

Appendix B: Eligibility Questionnaire

The effect of dietary calcium intake as compared to calcium supplementation on vascular and bone health in postmenopausal women

Screening Sheet

Name: Date:

		Study Exclusion	
A.	**Personal Information**		
1.	Current Age _____ (< 55 years old)	Yes ❒	No ❒
2.	Age at menopause _____ (≤ 3 years ago)	Yes ❒	No ❒
3.	Height (cm) _____ Weight (kg) _____ BMI _____ (<20 kg/m^2 or >30 kg/m^2)	Yes ❒	No ❒
4.	Smoker (in the last 5 years)	Yes ❒	No ❒
5.	Alcohol (≥ 3 units per day)	Yes ❒	No ❒
B.	**Medical Conditions**		
1.	Atrial Fibrillation	Yes ❒	No ❒
2.	Documented Atherosclerosis	Yes ❒	No ❒
3.	Hypertension (systolic BP >150mmHg ❒, diastolic BP >90mmHg ❒)	Yes ❒	No ❒
4.	Hyperparathyroidism	Yes ❒	No ❒
5.	Rheumatoid Arthritis	Yes ❒	No ❒
6.	Urinary Tract Lithiasis (documented or not)	Yes ❒	No ❒
7.	Diabetes	Yes ❒	No ❒
8.	Gestational: diabetes ❒, hypertension ❒, pre-eclampsia ❒	Yes ❒	No ❒
C.	**Medications and supplements**		
1.	Bone active drugs in the last 3 years (oral glucocorticoids ❒, bisphosphonates ❒, SERMs ❒, denosumab ❒, teriparatide ❒, calcitonin ❒, HRT [excluding vaginal preparations] ❒)	Yes ❒	No ❒
2.	Anti-hypertensives	Yes ❒	No ❒
3.	Chronic NSAID use	Yes ❒	No ❒
4.	Calcium in supplemental form (in the last 2 months) (___mg supplement ❒, calcium-containing antacids ❒, multivitamin ❒)	Yes ❒	No ❒
5.	Vitamin D in supplemental form (in the last 2 months) (___IU supplement ❒, fish liver oil ❒)	Yes ❒	No ❒
D.	**FRAX Calculation**		
1.	Previous fracture after age 40 ❒ (vertebral ❒, spontaneous ❒, or low-trauma ❒)		
2.	Parental hip fracture ❒		
	FRAX Score ≥ 20	Yes ❒	No ❒

Appendix C: International Physical Activity Questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

Appendix C: International Physical Activity Questionnaire

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

Appendix C: International Physical Activity Questionnaire

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

Appendix C: International Physical Activity Questionnaire

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

Appendix C: International Physical Activity Questionnaire

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

Appendix D: Food Frequency Questionnaire

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

Food item	**Never**	**Servings per** **month**	**week**	**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) 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)

Appendix D: Food Frequency Questionnaire

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 = 112g)*	□	_____	_____	_____	□ 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)*	□	_____	_____	_____	g (0.5 oz) g (1.0 oz) g (2.0 oz)
Firm or processed cheese *(including in sandwich or mixed dish)* *(blue, fontina, cheddar, Swiss, gouda, colby, edam, provolone, brick)*	□	_____	_____	_____	g (0.5 oz) g (1.0 oz) 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

Appendix D: Food Frequency Questionnaire

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

Appendix D: Food Frequency Questionnaire

Food item	Never	Servings per month	Servings per week	Servings per day	Serving size
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) 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) □ 112 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

Appendix D: Food Frequency Questionnaire

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)		

Appendix E: Vascular and Anthropometric Measurements Datasheet

CALCIUM
UNE ÉTUDE SUR L'IMPACT DU CALCIUM SUR LA SANTÉ VASCULAIRE DES FEMMES

Worksheet

Baseline visit ❒
6-month visit ❒
12-month visit ❒

Subject ID:
Birthdate:
(mm/dd/yyy)

Date:
Time:

1. Screening

	Yes	No
Strenuous physical activity in the last 48 hours?		
Consumed alcohol in the last 48 hours?		
Eaten in the last 12 hours?		
Tea or coffee in the last 12 hours?		
Taken supplements this morning?		

2. Anthropometric measurements

Reading	1	2	3	Repeat/Avg
Height (cm)				
Weight (kg)				
Waist circumference (cm)				
Hip circumference (cm)				
Body fat (%)	Std:	Ath:		

3. BPtru measurements

Reading	Left Arm	Right Arm	5 x Average	Standing
Brachial BP				

4. Resting hemodynamic measurements

Reading	1 (discard)	2	3
Brachial BP			
Average	XXXX		

5. Vascular resting hemodynamic measurements

Reading	1	2
Central BP SBP/DBP(MAP)PP		
AP		
Alx		
Alx (HR Corr.) HR		
crPWV HR		
cfPWV HR		

6. Vascular anthropometric measurements

Carotid (cm)	
Radial (cm)	
Femoral (cm)	

References

1. WorldHealthOrganisation(WHO). Cardiovascular disease Fact sheets. http://www.who.int/mediacentre/factsheets/fs317/en/. Accessed 5 February, 2017.
2. (PHAC) TPHAoC. Chronic Disease And Injury Indicator Framework ; Quick Stats, 2016 Edition http://www.phac-aspc.gc.ca/publicat/hpcdp-pspmc/36-8/assets/pdf/ar-04-engpdf. Accessed 5 Feb, 2017.
3. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med.* 2014;5(8):927-946.
4. 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.
5. Ding FH, Fan WX, Zhang RY, Zhang Q, Li Y, Wang JG. Validation of the noninvasive assessment of central blood pressure by the SphygmoCor and Omron devices against the invasive catheter measurement. *Am J Hypertens.* 2011;24(12):1306-1311.
6. Michaelsson K, Melhus H, Warensjo 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.
7. Bolland MJ, Avenell A, Baron JA, et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *Bmj.* 2010;341:c3691.
8. 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.
9. McGill HC, Jr., McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation.* 2008;117(9):1216-1227.
10. Elias-Smale SE, Kavousi M, Verwoert GC, et al. Common carotid intima-media thickness in cardiovascular risk stratification of older people: the Rotterdam Study. *Eur J Prev Cardiol.* 2012;19(4):698-705.
11. StatisticsCanada. Persons diagnosed by a health professional as having certain conditions, prevalence of these conditions and distribution of cases, by age group, Canada, 2009. http://www.statcan.gc.ca/pub/89-503-x/2010001/article/11543/tbl/tbl007-enghtm. Accessed 5 February, 2017.
12. Hage FG, Oparil S. Ovarian hormones and vascular disease. *Curr Opin Cardiol.* 2013;28(4):411-416.
13. Canada PHAo. Tracking Heart Disease and Stroke in Canada. http://www.phac-aspc.gc.ca/publicat/2009/cvd-avc/pdf/cvd-avs-2009-engpdf. 2009.
14. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation.* 2013;127(1):e6-e245.
15. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. *JAMA Cardiol.* 2016;1(7):767-776.

16. Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. *Menopause.* 2012;19(10):1081-1087.
17. Lokkegaard E, Jovanovic Z, Heitmann BL, Keiding N, Ottesen B, Pedersen AT. The association between early menopause and risk of ischaemic heart disease: influence of Hormone Therapy. *Maturitas.* 2006;53(2):226-233.
18. Cooper GS, Ephross SA, Weinberg CR, Baird DD, Whelan EA, Sandler DP. Menstrual and reproductive risk factors for ischemic heart disease. *Epidemiology.* 1999;10(3):255-259.
19. Hu FB, Grodstein F, Hennekens CH, et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med.* 1999;159(10):1061-1066.
20. Hong JS, Yi SW, Kang HC, et al. Age at menopause and cause-specific mortality in South Korean women: Kangwha Cohort Study. *Maturitas.* 2007;56(4):411-419.
21. Mondul AM, Rodriguez C, Jacobs EJ, Calle EE. Age at natural menopause and cause-specific mortality. *Am J Epidemiol.* 2005;162(11):1089-1097.
22. Jacobsen BK, Heuch I, Kvale G. Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. *Am J Epidemiol.* 2003;157(10):923-929.
23. Ossewaarde ME, Bots ML, Verbeek AL, et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology.* 2005;16(4):556-562.
24. Lisabeth LD, Beiser AS, Brown DL, Murabito JM, Kelly-Hayes M, Wolf PA. Age at natural menopause and risk of ischemic stroke: the Framingham heart study. *Stroke.* 2009;40(4):1044-1049.
25. Agrinier N, Cournot M, Dallongeville J, et al. Menopause and modifiable coronary heart disease risk factors: a population based study. *Maturitas.* 2010;65(3):237-243.
26. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab.* 2003;88(6):2404-2411.
27. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med.* 2003;163(4):427-436.
28. Toth MJ, Tchernof A, Sites CK, Poehlman ET. Effect of menopausal status on body composition and abdominal fat distribution. *Int J Obes Relat Metab Disord.* 2000;24(2):226-231.
29. Poehlman ET, Toth MJ, Gardner AW. Changes in energy balance and body composition at menopause: a controlled longitudinal study. *Ann Intern Med.* 1995;123(9):673-675.
30. Li Z, McNamara JR, Fruchart JC, et al. Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. *J Lipid Res.* 1996;37(9):1886-1896.
31. Stevenson JC, Crook D, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. *Atherosclerosis.* 1993;98(1):83-90.
32. Lindheim SR, Buchanan TA, Duffy DM, et al. Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. *J Soc Gynecol Investig.* 1994;1(2):150-154.
33. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev.* 2002;23(1):90-119.

34. Centurion OA. Carotid Intima-Media Thickness as a Cardiovascular Risk Factor and Imaging Pathway of Atherosclerosis. *Crit Pathw Cardiol.* 2016;15(4):152-160.
35. Mack WJ, Slater CC, Xiang M, Shoupe D, Lobo RA, Hodis HN. Elevated subclinical atherosclerosis associated with oophorectomy is related to time since menopause rather than type of menopause. *Fertil Steril.* 2004;82(2):391-397.
36. Zhao Z, Wang H, Jessup JA, Lindsey SH, Chappell MC, Groban L. Role of estrogen in diastolic dysfunction. *Am J Physiol Heart Circ Physiol.* 2014;306(5):H628-640.
37. Wang H, Jessup JA, Lin MS, Chagas C, Lindsey SH, Groban L. Activation of GPR30 attenuates diastolic dysfunction and left ventricle remodelling in oophorectomized mRen2.Lewis rats. *Cardiovasc Res.* 2012;94(1):96-104.
38. Yung LM, Wong WT, Tian XY, et al. Inhibition of renin-angiotensin system reverses endothelial dysfunction and oxidative stress in estrogen deficient rats. *PLoS One.* 2011;6(3):e17437.
39. Yanes LL, Romero DG, Iliescu R, Zhang H, Davis D, Reckelhoff JF. Postmenopausal hypertension: role of the Renin-Angiotensin system. *Hypertension.* 2010;56(3):359-363.
40. Grodstein F, Stampfer MJ, Colditz GA, et al. Postmenopausal hormone therapy and mortality. *N Engl J Med.* 1997;336(25):1769-1775.
41. Grodstein F, Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. *Prog Cardiovasc Dis.* 1995;38(3):199-210.
42. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288(3):321-333.
43. Osteoporosis prevention, diagnosis, and therapy. *Jama.* 2001;285(6):785-795.
44. WHO Scientific Group on the Prevention and Management of Osteoporosis (2000 : Geneva S. "Prevention and management of osteoporosis : report of a WHO scientific group". 2003.
45. Berger C, Goltzman D, Langsetmo L, 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.
46. Kleerekoper M. Osteoporosis Overview. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*: John Wiley & Sons, Inc.; 2013:343-347.
47. Bittar EE, Bittar N. Reproductive endocrinology and biology. 1998.
48. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev.* 2000;21(2):115-137.
49. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest.* 2003;111(8):1221-1230.
50. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology.* 1999;140(9):4367-4370.
51. Charatcharoenwitthaya N, Khosla S, Atkinson EJ, McCready LK, Riggs BL. Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. *J Bone Miner Res.* 2007;22(5):724-729.
52. Jilka RL, Hangoc G, Girasole G, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. *Science.* 1992;257(5066):88-91.

53. Ammann P, Rizzoli R, Bonjour JP, et al. Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. *J Clin Invest.* 1997;99(7):1699-1703.
54. Drake MT, Clarke BL, Lewiecki EM. The Pathophysiology and Treatment of Osteoporosis. *Clin Ther.* 2015;37(8):1837-1850.
55. Gallagher JC, Young MM, Nordin BE. Effects of artificial menopause on plasma and urine calcium and phosphate. *Clin Endocrinol (Oxf).* 1972;1(1):57-64.
56. Gennari C, Agnusdei D, Nardi P, Civitelli R. Estrogen preserves a normal intestinal responsiveness to 1,25-dihydroxyvitamin D3 in oophorectomized women. *J Clin Endocrinol Metab.* 1990;71(5):1288-1293.
57. Khosla S, Melton LJ, 3rd, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? *J Bone Miner Res.* 2011;26(3):441-451.
58. McKane WR, Khosla S, Burritt MF, et al. Mechanism of renal calcium conservation with estrogen replacement therapy in women in early postmenopause--a clinical research center study. *J Clin Endocrinol Metab.* 1995;80(12):3458-3464.
59. Barrett KE, Boitano S, Susan MB. *Ganong's Review of Medical Physiology (23rd Edition) (23).* New York, USA, US: McGraw-Hill; 2010.
60. Hall JE, Guyton AC. *Guyton and Hall textbook of medical physiology.* 2011.
61. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev.* 2005;85(1):373-422.
62. Kearns AE, Khosla S, Kostenuik PJ. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev.* 2008;29(2):155-192.
63. Chen RA, Goodman WG. Role of the calcium-sensing receptor in parathyroid gland physiology. *Am J Physiol Renal Physiol.* 2004;286(6):F1005-1011.
64. Papaioannou A, Morin S, Cheung AM, et al. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. *Cmaj.* 2010;182(17):1864-1873.
65. Report Ib. Dietary Reference Intakes for Calcium and Vitamin D. https://www.nationalacademies.org/hmd/~/media/Files/Report Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin D and Calcium 2010 Report Briefpdf. Published Nov 2010.
66. Canada O. HOW MUCH CALCIUM DO WE NEED? http://www.osteoporosis.ca/osteoporosis-and-you/nutrition/calcium-requirements/. Accessed 6 February, 2017.
67. Bailey RL, Dodd KW, Goldman JA, et al. Estimation of total usual calcium and vitamin D intakes in the United States. *J Nutr.* 2010;140(4):817-822.
68. Garriguet D. Bone health: osteoporosis, calcium and vitamin D. *Health Rep.* 2011;22(3):7-14.
69. Reid IR, Schooler BA, Hannan SF, Ibbertson HK. THE ACUTE BIOCHEMICAL EFFECTS OF FOUR PROPRIETARY CALCIUM PREPARATIONS. *Australian and New Zealand Journal of Medicine.* 1986;16(2):193-197.
70. 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.

71. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354(7):669-683.
72. Warensjo E, Byberg L, Melhus H, et al. Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study. *Bmj.* 2011;342:d1473.
73. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, 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.
74. Kanis JA, Johansson H, Oden A, et al. A meta-analysis of milk intake and fracture risk: low utility for case finding. *Osteoporos Int.* 2005;16(7):799-804.
75. Lewis JR, Radavelli-Bagatini S, Rejnmark L, et al. 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.
76. Wang X, Chen H, Ouyang Y, et al. Dietary calcium intake and mortality risk from cardiovascular disease and all causes: a meta-analysis of prospective cohort studies. *BMC Med.* 2014;12:158.
77. 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.
78. Bolland MJ, Barber PA, Doughty RN, et al. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *Bmj.* 2008;336(7638):262-266.
79. Pentti K, Tuppurainen MT, Honkanen R, et al. Use of calcium supplements and the risk of coronary heart disease in 52-62-year-old women: The Kuopio Osteoporosis Risk Factor and Prevention Study. *Maturitas.* 2009;63(1):73-78.
80. Li K, Kaaks R, Linseisen J, Rohrmann S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg). *Heart.* 2012;98(12):920-925.
81. Yang B, Campbell PT, Gapstur SM, et al. Calcium intake and mortality from all causes, cancer, and cardiovascular disease: the Cancer Prevention Study II Nutrition Cohort. *Am J Clin Nutr.* 2016;103(3):886-894.
82. Xiao Q, Murphy RA, Houston DK, Harris TB, Chow WH, Park Y. Dietary and supplemental calcium intake and cardiovascular disease mortality: the National Institutes of Health-AARP diet and health study. *JAMA Intern Med.* 2013;173(8):639-646.
83. 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.
84. Wang L, Manson JE, Sesso HD. Calcium intake and risk of cardiovascular disease: a review of prospective studies and randomized clinical trials. *Am J Cardiovasc Drugs.* 2012;12(2):105-116.
85. Hsia J, Heiss G, Ren H, et al. Calcium/vitamin D supplementation and cardiovascular events. *Circulation.* 2007;115(7):846-854.
86. 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.

87. Paik JM, Curhan GC, Sun Q, et al. Calcium supplement intake and risk of cardiovascular disease in women. *Osteoporos Int.* 2014;25(8):2047-2056.
88. Adebamowo SN, Spiegelman D, Willett WC, Rexrode KM. Association between intakes of magnesium, potassium, and calcium and risk of stroke: 2 cohorts of US women and updated meta-analyses. *Am J Clin Nutr.* 2015;101(6):1269-1277.
89. Mursu J, Robien K, Harnack LJ, Park K, Jacobs DR, Jr. Dietary supplements and mortality rate in older women: the Iowa Women's Health Study. *Arch Intern Med.* 2011;171(18):1625-1633.
90. Al-Delaimy WK, Rimm E, Willett WC, Stampfer MJ, Hu FB. A prospective study of calcium intake from diet and supplements and risk of ischemic heart disease among men. *Am J Clin Nutr.* 2003;77(4):814-818.
91. Van Hemelrijck M, Michaelsson K, Linseisen J, Rohrmann S. Calcium intake and serum concentration in relation to risk of cardiovascular death in NHANES III. *PLoS One.* 2013;8(4):e61037.
92. Iso H, Stampfer MJ, Manson JE, et al. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. *Stroke.* 1999;30(9):1772-1779.
93. Tian DY, Tian J, Shi CH, et al. Calcium intake and the risk of stroke: an up-dated meta-analysis of prospective studies. *Asia Pac J Clin Nutr.* 2015;24(2):245-252.
94. 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.
95. Umesawa M, Iso H, Ishihara J, et al. Dietary calcium intake and risks of stroke, its subtypes, and coronary heart disease in Japanese: the JPHC Study Cohort I. *Stroke.* 2008;39(9):2449-2456.
96. Umesawa M, Iso H, Date C, et al. Dietary intake of calcium in relation to mortality from cardiovascular disease: the JACC Study. *Stroke.* 2006;37(1):20-26.
97. Reid IR, Bolland MJ, Avenell A, Grey A. Cardiovascular effects of calcium supplementation. *Osteoporos Int.* 2011;22(6):1649-1658.
98. Bristow SM, Gamble GD, Stewart A, Kalluru R, Horne AM, Reid IR. Acute effects of calcium citrate with or without a meal, calcium-fortified juice and a dairy product meal on serum calcium and phosphate: a randomised cross-over trial. *Br J Nutr.* 2015;113(10):1585-1594.
99. Bristow SM, Gamble GD, Stewart A, et al. Acute and 3-month effects of microcrystalline hydroxyapatite, calcium citrate and calcium carbonate on serum calcium and markers of bone turnover: a randomised controlled trial in postmenopausal women. *Br J Nutr.* 2014;112(10):1611-1620.
100. Salvi P. Pulse waves how vascular hemodynamics affects blood pressure. 2012; http://public.eblib.com/choice/publicfullrecord.aspx?p=973174.
101. Burt MG, Mangelsdorf BL, Srivastava D, Petersons CJ. Acute effect of calcium citrate on serum calcium and cardiovascular function. *J Bone Miner Res.* 2013;28(2):412-418.
102. Slinin Y, Blackwell T, Ishani A, Cummings SR, Ensrud KE. Serum calcium, phosphorus and cardiovascular events in post-menopausal women. *Int J Cardiol.* 2011;149(3):335-340.
103. 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.

104. Leifsson BG, Ahren B. Serum calcium and survival in a large health screening program. *J Clin Endocrinol Metab.* 1996;81(6):2149-2153.
105. 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.
106. Shaker JL, Deftos L. Calcium and Phosphate Homeostasis. In: De Groot LJ, Beck-Peccoz P, Chrousos G, et al., eds. *Endotext*. South Dartmouth MA: MDText.com, Inc.; 2000.
107. Bristow SM, Gamble GD, Pasch A, et al. Acute and 3-month effects of calcium carbonate on the calcification propensity of serum and regulators of vascular calcification: secondary analysis of a randomized controlled trial. *Osteoporos Int.* 2016;27(3):1209-1216.
108. Bristow SM, Gamble GD, Stewart A, Horne AM, Reid IR. Acute effects of calcium supplements on blood pressure and blood coagulation: secondary analysis of a randomised controlled trial in post-menopausal women. *The British journal of nutrition.* 2015;114(11):1868-1874.
109. Li JK-J. Dynamics of the vascular system. River Edge. *NJ: World Scientific.* 2004:23-24.
110. Nichols WW, O'Rourke, M. F., & McDonald, D. A. McDonald's blood flow in arteries: Theoretic, experimental, and clinical principles. London: Hodder Arnold. 2005.
111. P S. Pulse Waves: How Vascular Hemodynamics Affects Blood Pressure. *Milan, Italy: Springer Milan.* 2012.
112. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* 2005;25(5):932-943.
113. O'Rourke MF, Hashimoto J. Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol.* 2007;50(1):1-13.
114. Sun Z. Aging, arterial stiffness, and hypertension. *Hypertension.* 2015;65(2):252-256.
115. Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. *Circulation.* 2011;123(17):1900-1910.
116. Semba RD, Najjar SS, Sun K, Lakatta EG, Ferrucci L. Serum carboxymethyl-lysine, an advanced glycation end product, is associated with increased aortic pulse wave velocity in adults. *Am J Hypertens.* 2009;22(1):74-79.
117. Dao HH, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. *Cardiovasc Res.* 2005;66(2):307-317.
118. Fougere B, Boulanger E, Nourhashemi F, Guyonnet S, Cesari M. Chronic Inflammation: Accelerator of Biological Aging. *J Gerontol A Biol Sci Med Sci.* 2016.
119. Jain S, Khera R, Corrales-Medina VF, Townsend RR, Chirinos JA. "Inflammation and arterial stiffness in humans". *Atherosclerosis.* 2014;237(2):381-390.
120. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol.* 2004;24(5):969-974.
121. Wu J, Thabet SR, Kirabo A, et al. Inflammation and mechanical stretch promote aortic stiffening in hypertension through activation of p38 mitogen-activated protein kinase. *Circ Res.* 2014;114(4):616-625.
122. Safar M FE. Atherosclerosis, Large Arteries and Cardiovascular Risk. New York, USA: Karger. 2007.

123. Wilkinson IB, MacCallum H, Cockcroft JR, Webb DJ. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *Br J Clin Pharmacol.* 2002;53(2):189-192.
124. Joannides R, Richard V, Haefeli WE, et al. Role of nitric oxide in the regulation of the mechanical properties of peripheral conduit arteries in humans. *Hypertension.* 1997;30(6):1465-1470.
125. MacCarthy PA, Pegge NC, Prendergast BD, Shah AM, Groves PH. The physiological role of endogenous endothelin in the regulation of human coronary vasomotor tone. *J Am Coll Cardiol.* 2001;37(1):137-143.
126. McEniery CM, Qasem A, Schmitt M, Avolio AP, Cockcroft JR, Wilkinson IB. Endothelin-1 regulates arterial pulse wave velocity in vivo. *J Am Coll Cardiol.* 2003;42(11):1975-1981.
127. Mackenzie IS, Wilkinson IB, Cockcroft JR. Assessment of arterial stiffness in clinical practice. *QJM.* 2002;95(2):67-74.
128. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J.* 2006;27(21):2588-2605.
129. Kim DH, Braam B. Assessment of arterial stiffness using applanation tonometry. *Can J Physiol Pharmacol.* 2013;91(12):999-1008.
130. SphygmCor. http://atcormedical.com/healthcare-professionals/products/. Accessed January 2017.
131. Townsend RR, Wilkinson IB, Schiffrin EL, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension (Dallas, Tex : 1979).* 2015;66(3):698-722.
132. Sugawara J, Hayashi K, Yokoi T, Tanaka H. Age-associated elongation of the ascending aorta in adults. *JACC Cardiovasc Imaging.* 2008;1(6):739-748.
133. Weber T, Ammer M, Rammer M, et al. Noninvasive determination of carotid-femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. *J Hypertens.* 2009;27(8):1624-1630.
134. Huybrechts SA, Devos DG, Vermeersch SJ, et al. Carotid to femoral pulse wave velocity: a comparison of real travelled aortic path lengths determined by MRI and superficial measurements. *J Hypertens.* 2011;29(8):1577-1582.
135. Sugawara J, Hayashi K, Yokoi T, et al. Brachial-ankle pulse wave velocity: an index of central arterial stiffness? *J Hum Hypertens.* 2005;19(5):401-406.
136. 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.
137. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation.* 2010;121(4):505-511.
138. 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.
139. Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. *Am J Hypertens.* 2005;18(1 Pt 2):3S-10S.

140. Salvi P, Safar ME, Labat C, Borghi C, Lacolley P, Benetos A. Heart disease and changes in pulse wave velocity and pulse pressure amplification in the elderly over 80 years: the PARTAGE Study. *J Hypertens.* 2010;28(10):2127-2133.
141. Segers P, Mahieu D, Kips J, et al. Amplification of the pressure pulse in the upper limb in healthy, middle-aged men and women. *Hypertension.* 2009;54(2):414-420.
142. McEniery CM, Yasmin, McDonnell B, et al. Central pressure: variability and impact of cardiovascular risk factors: the Anglo-Cardiff Collaborative Trial II. *Hypertension.* 2008;51(6):1476-1482.
143. Protogerou AD, Stergiou GS, Vlachopoulos C, Blacher J, Achimastos A. The effect of antihypertensive drugs on central blood pressure beyond peripheral blood pressure. Part II: Evidence for specific class-effects of antihypertensive drugs on pressure amplification. *Curr Pharm Des.* 2009;15(3):272-289.
144. Williams B, Lacy PS, Thom SM, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation.* 2006;113(9):1213-1225.
145. Dahlof B, Sever PS, Poulter NR, et al. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet.* 2005;366(9489):895-906.
146. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol.* 2000;525 Pt 1:263-270.
147. Rosenbaum D, Giral P, Chapman J, et al. Radial augmentation index is a surrogate marker of atherosclerotic burden in a primary prevention cohort. *Atherosclerosis.* 2013;231(2):436-441.
148. Chirinos JA, Zambrano JP, Chakko S, et al. Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease. *Hypertension.* 2005;45(5):980-985.
149. Weber T, Auer J, O'Rourke M F, et al. Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. *Eur Heart J.* 2005;26(24):2657-2663.
150. Fischer-Rasokat U, Brenck F, Zeiher AM, Spyridopoulos I. Radial augmentation index unmasks premature coronary artery disease in younger males. *Blood Press Monit.* 2009;14(2):59-67.
151. Guelen I, Mattace-Raso FU, van Popele NM, et al. Aortic stiffness and the balance between cardiac oxygen supply and demand: the Rotterdam Study. *J Hypertens.* 2008;26(6):1237-1243.
152. Di Micco L, Salvi P, Bellasi A, Sirico ML, Di Iorio B. Subendocardial viability ratio predicts cardiovascular mortality in chronic kidney disease patients. *Blood Purif.* 2013;36(1):26-28.
153. Mosimann K, Jacomella V, Thalhammer C, et al. Severity of peripheral arterial disease is associated with aortic pressure augmentation and subendocardial viability ratio. *J Clin Hypertens (Greenwich).* 2012;14(12):855-860.

154. Tsiachris D, Tsioufis C, Syrseloudis D, et al. Subendocardial viability ratio as an index of impaired coronary flow reserve in hypertensives without significant coronary artery stenoses. *J Hum Hypertens.* 2012;26(1):64-70.
155. Aortic pulse pressure waveform. https://www.hindawi.com/journals/ijvm/2012/903107/fig1/. Accessed_28_December_2017.
156. Baulmann J, Schillings U, Rickert S, et al. A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *J Hypertens.* 2008;26(3):523-528.
157. Rajzer MW, Wojciechowska W, Klocek M, Palka I, Brzozowska-Kiszka M, Kawecka-Jaszcz K. Comparison of aortic pulse wave velocity measured by three techniques: Complior, SphygmoCor and Arteriograph. *J Hypertens.* 2008;26(10):2001-2007.
158. Jatoi NA, Mahmud A, Bennett K, Feely J. Assessment of arterial stiffness in hypertension: comparison of oscillometric (Arteriograph), piezoelectronic (Complior) and tonometric (SphygmoCor) techniques. *J Hypertens.* 2009;27(11):2186-2191.
159. Hirata K, Kojima I, Momomura S. Noninvasive estimation of central blood pressure and the augmentation index in the seated position: a validation study of two commercially available methods. *J Hypertens.* 2013;31(3):508-515; discussion 515.
160. Sharman JE, Lim R, Qasem AM, et al. Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension.* 2006;47(6):1203-1208.
161. Laugesen E, Rossen NB, Peters CD, et al. Assessment of central blood pressure in patients with type 2 diabetes: a comparison between SphygmoCor and invasively measured values. *Am J Hypertens.* 2014;27(2):169-176.
162. Wilkinson IB, Fuchs SA, Jansen IM, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens.* 1998;16(12 Pt 2):2079-2084.
163. Wilkinson IB, McEniery CM, Schillaci G, et al. ARTERY Society guidelines for validation of non-invasive haemodynamic measurement devices: Part 1, arterial pulse wave velocity. *Artery Research.* 2010;4(2):34-40.
164. Yaron M, Roach V, Izkhakov E, et al. 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.
165. 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.
166. Uemura H, Katsuura-Kamano S, Yamaguchi M, Nakamoto M, Hiyoshi M, Arisawa K. Association between dietary calcium intake and arterial stiffness according to dietary vitamin D intake in men. *Br J Nutr.* 2014;112(8):1333-1340.
167. Gray H, Standring S, Ellis H, Berkovitz BKB. *Gray's anatomy : the anatomical basis of clinical practice.* 2005.
168. Bauer M, Caviezel S, Teynor A, Erbel R, Mahabadi AA, Schmidt-Trucksass A. Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. *Swiss Med Wkly.* 2012;142:w13705.
169. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature.* 2011;473(7347):317-325.

170. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation.* 1986;74(6):1399-1406.
171. Wong M, Edelstein J, Wollman J, Bond MG. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. *Arterioscler Thromb.* 1993;13(4):482-486.
172. Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand J. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol.* 1991;11(6):565-577.
173. Allan PL, Mowbray PI, Lee AJ, Fowkes FG. Relationship between carotid intima-media thickness and symptomatic and asymptomatic peripheral arterial disease. The Edinburgh Artery Study. *Stroke.* 1997;28(2):348-353.
174. Bots ML, Baldassarre D, Simon A, et al. Carotid intima-media thickness and coronary atherosclerosis: weak or strong relations? *Eur Heart J.* 2007;28(4):398-406.
175. Bots ML, Hofman A, De Jong PT, Grobbee DE. Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery. The Rotterdam Study. *Ann Epidemiol.* 1996;6(2):147-153.
176. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. *Arterioscler Thromb.* 1994;14(12):1885-1891.
177. Howard G, Burke GL, Evans GW, et al. Relations of intimal-medial thickness among sites within the carotid artery as evaluated by B-mode ultrasound. ARIC Investigators. Atherosclerosis Risk in Communities. *Stroke.* 1994;25(8):1581-1587.
178. Ogata T, Yasaka M, Yamagishi M, Seguchi O, Nagatsuka K, Minematsu K. Atherosclerosis found on carotid ultrasonography is associated with atherosclerosis on coronary intravascular ultrasonography. *J Ultrasound Med.* 2005;24(4):469-474.
179. Tzou WS, Douglas PS, Srinivasan SR, et al. Distribution and predictors of carotid intima-media thickness in young adults. *Prev Cardiol.* 2007;10(4):181-189.
180. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr.* 2008;21(2):93-111; quiz 189-190.
181. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med.* 1999;340(1):14-22.
182. Howard G, Sharrett AR, Heiss G, et al. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. *Stroke.* 1993;24(9):1297-1304.
183. Simon A, Gariepy J, Chironi G, Megnien JL, Levenson J. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. *J Hypertens.* 2002;20(2):159-169.
184. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B. Incident coronary events and case fatality in relation to common carotid intima-media thickness. *J Intern Med.* 2005;257(5):430-437.

185. Denarie N, Gariepy J, Chironi G, et al. Distribution of ultrasonographically-assessed dimensions of common carotid arteries in healthy adults of both sexes. *Atherosclerosis.* 2000;148(2):297-302.
186. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke.* 2006;37(1):87-92.
187. Bennett PC, Gill PS, Silverman S, Blann AD, Lip GY. Ethnic differences in common carotid intima-media thickness, and the relationship to cardiovascular risk factors and peripheral arterial disease: the Ethnic-Echocardiographic Heart of England Screening Study. *Qjm.* 2011;104(3):245-254.
188. Mackinnon AD, Jerrard-Dunne P, Porteous L, Markus HS. Carotid intima-media thickness is greater but carotid plaque prevalence is lower in black compared with white subjects. *AJNR Am J Neuroradiol.* 2010;31(10):1951-1955.
189. Du HW, Li JY, He Y. Glycemic and blood pressure control in older patients with hypertension and diabetes: association with carotid atherosclerosis. *J Geriatr Cardiol.* 2011;8(1):24-30.
190. Ebrahim S, Papacosta O, Whincup P, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke.* 1999;30(4):841-850.
191. Manios E, Tsivgoulis G, Koroboki E, et al. Impact of prehypertension on common carotid artery intima-media thickness and left ventricular mass. *Stroke.* 2009;40(4):1515-1518.
192. de Freitas EV, Brandao AA, Pozzan R, Magalhies ME, Castier M, Brandao AP. Study of the intima-media thickening in carotid arteries of healthy elderly with high blood pressure and elderly with high blood pressure and dyslipidemia. *Clin Interv Aging.* 2008;3(3):525-534.
193. Wagenknecht LE, D'Agostino RB, Jr., Haffner SM, Savage PJ, Rewers M. Impaired glucose tolerance, type 2 diabetes, and carotid wall thickness: the Insulin Resistance Atherosclerosis Study. *Diabetes Care.* 1998;21(11):1812-1818.
194. Temelkova-Kurktschiev TS, Koehler C, Leonhardt W, et al. Increased intimal-medial thickness in newly detected type 2 diabetes: risk factors. *Diabetes Care.* 1999;22(2):333-338.
195. Brohall G, Oden A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. *Diabet Med.* 2006;23(6):609-616.
196. Hisamatsu T, Miura K, Arima H, et al. Smoking, Smoking Cessation, and Measures of Subclinical Atherosclerosis in Multiple Vascular Beds in Japanese Men. *J Am Heart Assoc.* 2016;5(9).
197. Koskinen J, Kahonen M, Viikari JS, et al. Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study. *Circulation.* 2009;120(3):229-236.
198. Peters SA, Lind L, Palmer MK, et al. Increased age, high body mass index and low HDL-C levels are related to an echolucent carotid intima-media: the METEOR study. *J Intern Med.* 2012;272(3):257-266.

199. Huang F, Yang Z, Xu B, et al. Both serum apolipoprotein B and the apolipoprotein B/apolipoprotein A-I ratio are associated with carotid intima-media thickness. *PLoS One.* 2013;8(1):e54628.
200. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation.* 1998;97(18):1837-1847.
201. Nambi V, Chambless L, Folsom AR, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. *J Am Coll Cardiol.* 2010;55(15):1600-1607.
202. Baldassarre D, Hamsten A, Veglia F, et al. Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study. *J Am Coll Cardiol.* 2012;60(16):1489-1499.
203. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2010;56(25):e50-103.
204. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation.* 2002;106(25):3143-3421.
205. de la Sierra A, Zamorano JL, Ruilope LM. Application of hypertension guidelines in clinical practice: implementation of the 2007 ESH/ESC European practice Guidelines in Spain. *J Hypertens Suppl.* 2009;27(3):S27-32.
206. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation.* 2007;115(4):459-467.
207. Chambless LE, Heiss G, Folsom AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol.* 1997;146(6):483-494.
208. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation.* 1997;96(5):1432-1437.
209. Crouse JR, 3rd, Byington RP, Bond MG, et al. Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries (PLAC-II). *Am J Cardiol.* 1995;75(7):455-459.
210. de Groot E, Jukema JW, Montauban van Swijndregt AD, et al. B-mode ultrasound assessment of pravastatin treatment effect on carotid and femoral artery walls and its correlations with coronary arteriographic findings: a report of the Regression Growth Evaluation Statin Study (REGRESS). *J Am Coll Cardiol.* 1998;31(7):1561-1567.
211. MacMahon S, Sharpe N, Gamble G, et al. Effects of lowering average of below-average cholesterol levels on the progression of carotid atherosclerosis: results of the LIPID Atherosclerosis Substudy. LIPID Trial Research Group. *Circulation.* 1998;97(18):1784-1790.

212. Furberg CD, Adams HP, Jr., Applegate WB, et al. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Asymptomatic Carotid Artery Progression Study (ACAPS) Research Group. *Circulation.* 1994;90(4):1679-1687.
213. Mercuri M, Bond MG, Sirtori CR, et al. Pravastatin reduces carotid intima-media thickness progression in an asymptomatic hypercholesterolemic mediterranean population: the Carotid Atherosclerosis Italian Ultrasound Study. *Am J Med.* 1996;101(6):627-634.
214. Hedblad B, Wikstrand J, Janzon L, Wedel H, Berglund G. Low-dose metoprolol CR/XL and fluvastatin slow progression of carotid intima-media thickness: Main results from the Beta-Blocker Cholesterol-Lowering Asymptomatic Plaque Study (BCAPS). *Circulation.* 2001;103(13):1721-1726.
215. Pitt B, Byington RP, Furberg CD, et al. Effect of amlodipine on the progression of atherosclerosis and the occurrence of clinical events. PREVENT Investigators. *Circulation.* 2000;102(13):1503-1510.
216. Lonn E, Yusuf S, Dzavik V, et al. Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation.* 2001;103(7):919-925.
217. Kawasumi M, Tanaka Y, Uchino H, et al. Strict glycemic control ameliorates the increase of carotid IMT in patients with type 2 diabetes. *Endocr J.* 2006;53(1):45-50.
218. Mita T, Watada H, Shimizu T, et al. Nateglinide reduces carotid intima-media thickening in type 2 diabetic patients under good glycemic control. *Arterioscler Thromb Vasc Biol.* 2007;27(11):2456-2462.
219. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis.* 2012;34(4):290-296.
220. Li S, Na L, Li Y, et al. Long-term calcium supplementation may have adverse effects on serum cholesterol and carotid intima-media thickness in postmenopausal women: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr.* 2013;98(5):1353-1359.
221. Anderson TJ, Gregoire J, Hegele RA, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol.* 2013;29(2):151-167.
222. Kampoli AM, Tousoulis D, Antoniades C, Siasos G, Stefanadis C. Biomarkers of premature atherosclerosis. *Trends Mol Med.* 2009;15(7):323-332.
223. Rogoveanu OC, Mogosanu GD, Bejenaru C, et al. Effects of Calcium Fructoborate on Levels of C-Reactive Protein, Total Cholesterol, Low-Density Lipoprotein, Triglycerides, IL-1beta, IL-6, and MCP-1: a Double-blind, Placebo-controlled Clinical Study. *Biol Trace Elem Res.* 2015;163(1-2):124-131.
224. Reid IR, Mason B, Horne A, et al. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. *Am J Med.* 2002;112(5):343-347.
225. Militaru C, Donoiu I, Craciun A, Scorei ID, Bulearca AM, Scorei RI. Oral resveratrol and calcium fructoborate supplementation in subjects with stable angina pectoris: effects on lipid profiles, inflammation markers, and quality of life. *Nutrition.* 2013;29(1):178-183.

226. Reid IR. Effects of calcium supplementation on circulating lipids: potential pharmacoeconomic implications. *Drugs Aging.* 2004;21(1):7-17.
227. Trautvetter U, Ditscheid B, Kiehntopf M, Jahreis G. A combination of calcium phosphate and probiotics beneficially influences intestinal lactobacilli and cholesterol metabolism in humans. *Clin Nutr.* 2012;31(2):230-237.
228. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *Faseb j.* 2000;14(9):1132-1138.
229. Li S, Li Y, Ning H, et al. Calcium supplementation increases circulating cholesterol by reducing its catabolism via GPER and TRPC1-dependent pathway in estrogen deficient women. *Int J Cardiol.* 2013;168(3):2548-2560.
230. Ma KY, Yang N, Jiao R, et al. Dietary calcium decreases plasma cholesterol by down-regulation of intestinal Niemann-Pick C1 like 1 and microsomal triacylglycerol transport protein and up-regulation of CYP7A1 and ABCG 5/8 in hamsters. *Mol Nutr Food Res.* 2011;55(2):247-258.
231. Torres MR, Francischetti EA, Genelhu V, Sanjuliani AF. Effect of a high-calcium energy-reduced diet on abdominal obesity and cardiometabolic risk factors in obese Brazilian subjects. *Int J Clin Pract.* 2010;64(8):1076-1083.
232. Smilowitz JT, Wiest MM, Teegarden D, Zemel MB, German JB, Van Loan MD. Dietary fat and not calcium supplementation or dairy product consumption is associated with changes in anthropometrics during a randomized, placebo-controlled energy-restriction trial. *Nutr Metab (Lond).* 2011;8:67.
233. 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.
234. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet.* 2002;360(9349):1903-1913.
235. Kannel WB. Blood pressure as a cardiovascular risk factor: Prevention and treatment. *JAMA.* 1996;275(20):1571-1576.
236. O'Shea PM, Griffin TP, Fitzgibbon M. Hypertension: The role of biochemistry in the diagnosis and management. *Clin Chim Acta.* 2017;465:131-143.
237. Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. *Circulation.* 2000;101(3):329-335.
238. van Mierlo LAJ, Arends LR, Streppel MT, et al. Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens.* 2006;20(8):571-580.
239. 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. *American Journal of Hypertension.* 1999;12(1):84-92.
240. Dickinson HO, Nicolson DJ, Cook JV, et al. Calcium supplementation for the management of primary hypertension in adults. *Cochrane Database Syst Rev.* 2006(2):Cd004639.
241. Reid IR, Ames R, Mason B, et al. 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.

242. Reid IR, Horne A, Mason B, Ames R, Bava U, Gamble GD. Effects of calcium supplementation on body weight and blood pressure in normal older women: a randomized controlled trial. *J Clin Endocrinol Metab.* 2005;90(7):3824-3829.
243. Margolis KL, Ray RM, Van Horn L, et al. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. *Hypertension.* 2008;52(5):847-855.
244. 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.
245. Cappuccio FP, Elliott P, Allender PS, Pryer J, Follman DA, Cutler JA. Epidemiologic association between dietary calcium intake and blood pressure: a meta-analysis of published data. *Am J Epidemiol.* 1995;142(9):935-945.
246. Schröder H, Schmelz E, Marrugat J. Relationship between diet and blood pressure in a representative Mediterranean population. *European Journal of Nutrition.* 2002;41(4):161-167.
247. Townsend MS, Fulgoni VL, Stern JS, Adu-Afarwuah S, McCarron DA. Low mineral intake is associated with high systolic blood pressure in the Third and Fourth National Health and Nutrition Examination Surveys*Could we all be right? *American Journal of Hypertension.* 2005;18(2):261-269.
248. Hajjar IM, Grim CE, Kotchen TA. Dietary Calcium Lowers the Age-Related Rise in Blood Pressure in the United States: The NHANES III Survey. *The Journal of Clinical Hypertension.* 2003;5(2):122-126.
249. Zemel MB. Calcium Modulation of Hypertension and Obesity: Mechanisms and Implications. *Journal of the American College of Nutrition.* 2001;20(sup5):428S-435S.
250. Resnick LM. The role of dietary calcium in hypertension: a hierarchical overview. *Am J Hypertens.* 1999;12(1 Pt 1):99-112.
251. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation.* 1997;96(11):4095-4103.
252. Libby P, Ridker PM. Novel inflammatory markers of coronary risk: theory versus practice. *Circulation.* 1999;100(11):1148-1150.
253. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med.* 2008;359(21):2195-2207.
254. Blann AD, Seigneur M, Steiner M, Boisseau MR, McCollum CN. Circulating endothelial cell markers in peripheral vascular disease: relationship to the location and extent of atherosclerotic disease. *Eur J Clin Invest.* 1997;27(11):916-921.
255. Blann AD, Miller JP, McCollum CN. von Willebrand factor and soluble E-selectin in the prediction of cardiovascular disease progression in hyperlipidaemia. *Atherosclerosis.* 1997;132(2):151-156.
256. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. *Ann Intern Med.* 1993;118(12):956-963.
257. Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *Jama.* 2005;294(14):1799-1809.

258. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. *Public Health Nutr.* 2010;13(9):1395-1402.
259. 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.
260. Hunter DC, Brown R, Green T, et al. Changes in markers of inflammation, antioxidant capacity and oxidative stress in smokers following consumption of milk, and milk supplemented with fruit and vegetable extracts and vitamin C. *Int J Food Sci Nutr.* 2012;63(1):90-102.
261. Nestel PJ, Pally S, MacIntosh GL, et al. Circulating inflammatory and atherogenic biomarkers are not increased following single meals of dairy foods. *Eur J Clin Nutr.* 2012;66(1):25-31.
262. 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.
263. van Meijl LE, Mensink RP. Effects of low-fat dairy consumption on markers of low-grade systemic inflammation and endothelial function in overweight and obese subjects: an intervention study. *Br J Nutr.* 2010;104(10):1523-1527.
264. 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.
265. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care.* 2007;30(4):980-986.
266. Gagnon C, Daly RM, Carpentier A, et al. 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.
267. Neyestani TR, Nikooyeh B, Alavi-Majd H, et al. 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.
268. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA.* 2013;310(20):2191-2194.
269. International_Osteoporosis_Foundation. Facts_and_Stattistics_about_Osteoporosis. https://www.iofbonehealth.org/facts-statistics - category-23. Accessed_21January_2018.
270. Institute_of_Medicine. IOM_Recommended_Dietary_Allowance_Calcium
. https://odsodnihgov/factsheets/Calcium-HealthProfessional/ - h2. Accessed_21January2018.
271. FRAX Score assessment. https://www.shefac.uk/FRAX/toolaspx?country=19. Accessed 7 February, 2017.
272. Ong A. 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, Canada.*

273. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc Sport.* 2000;71(2 Suppl):S114-120.
274. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* 2003;35(8):1381-1395.
275. International Physical Activity Questionnaire (2005) Guidelines for the data processing and analysis of the International Physical Activity Questionnaire – short and long forms. https://sites.google.com/site/theipaq/. Accessed 7 February, 2017.
276. Stadiometer.com. The seca 242 Ultimate Digital Height Measuring Device. http://www.stadiometer.com/242html. Accessed 5 May, 2017.
277. TanitaCorp. TBF-310GS/Total Body ComposiTion Analyzer. http://mediatanita.com/data/product-brochures/TBF-310GSpdf?rev=A5A0. Accessed 5 May, 2017.
278. The Canadian Physical Activity, Fitness and Lifestyle Approach Supplement to the third edition. http://www.provincialfitnessunit.ca/media/uploads/CPAFLA_Insert_Package_August_2010pdf. Accessed 8 February, 2017.
279. Daskalopoulou SS, Rabi DM, Zarnke KB, 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.
280. Ho SS. Current status of carotid ultrasound in atherosclerosis. *Quantitative imaging in medicine and surgery.* 2016;6(3):285-296.
281. 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.
282. Canada'sFoodGuide. Recommended Number of Food Guide Servings per Day http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit-engphp. Accessed 16 February, 2017.
283. OsteoporosisCanada. Calcium: An Important Nutrient that Builds Stronger Bones. http://www.osteoporosis.ca/osteoporosis-and-you/nutrition/calcium-requirements/. Accessed 15 February, 2017.
284. 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.
285. Kesse-Guyot E, Vergnaud AC, Fezeu L, et al. Associations between dietary patterns and arterial stiffness, carotid artery intima-media thickness and atherosclerosis. *Eur J Cardiovasc Prev Rehabil.* 2010;17(6):718-724.
286. Soares PA, Kovacs C, Moreira P, Saleh MH, Magnoni D, Faintuch J. Is intake of vitamin D and calcium important for cardiovascular health in elderly obese patients? *Obes Surg.* 2012;22(3):437-444.
287. Masley SC, Roetzheim R, Masley LV, McNamara T, Schocken DD. Emerging risk factors as markers for carotid intima media thickness scores. *J Am Coll Nutr.* 2015;34(2):100-107.

288. Ong AM, Weiler HA, Wall M, et al. 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.
289. Choi SJ, Yeum KJ, Park SJ, Choi B, Joo NS. Dietary calcium and Framingham Risk Score in vitamin D deficient male (KNHANES 2009-2011). *Yonsei Med J.* 2015;56(3):845-852.
290. van Mierlo LA, Arends LR, Streppel MT, et al. Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens.* 2006;20(8):571-580.
291. 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 Pt 1):84-92.
292. MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet.* 1990;335(8692):765-774.
293. Lawes CM, Bennett DA, Lewington S, Rodgers A. Blood pressure and coronary heart disease: a review of the evidence. *Semin Vasc Med.* 2002;2(4):355-368.
294. Lawes CM, Bennett DA, Feigin VL, Rodgers A. Blood pressure and stroke: an overview of published reviews. *Stroke.* 2004;35(3):776-785.
295. Roman MJ, Devereux RB, Kizer JR, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension.* 2007;50(1):197-203.
296. Pini R, Cavallini MC, Palmieri V, et al. Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. *J Am Coll Cardiol.* 2008;51(25):2432-2439.
297. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J.* 2010;31(15):1865-1871.
298. Safar ME, Blacher J, Pannier B, et al. Central pulse pressure and mortality in end-stage renal disease. *Hypertension.* 2002;39(3):735-738.
299. Miura K, Dyer AR, Greenland P, et al. Pulse pressure compared with other blood pressure indexes in the prediction of 25-year cardiovascular and all-cause mortality rates: The Chicago Heart Association Detection Project in Industry Study. *Hypertension.* 2001;38(2):232-237.
300. Sesso HD, Stampfer MJ, Rosner B, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in Men. *Hypertension.* 2000;36(5):801-807.
301. Benetos A, Gautier S, Labat C, et al. Mortality and cardiovascular events are best predicted by low central/peripheral pulse pressure amplification but not by high blood pressure levels in elderly nursing home subjects: the PARTAGE (Predictive Values of Blood Pressure and Arterial Stiffness in Institutionalized Very Aged Population) study. *J Am Coll Cardiol.* 2012;60(16):1503-1511.
302. Lewington S, Whitlock G, Clarke R, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* 2007;370(9602):1829-1839.

303. Blood pressure, cholesterol, and stroke in eastern Asia. Eastern Stroke and Coronary Heart Disease Collaborative Research Group. *Lancet.* 1998;352(9143):1801-1807.
304. Kelly KA, Gimble JM. 1,25-Dihydroxy vitamin D3 inhibits adipocyte differentiation and gene expression in murine bone marrow stromal cell clones and primary cultures. *Endocrinology.* 1998;139(5):2622-2628.
305. 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.
306. De Oliveira ESER, Foster D, McGee Harper M, et al. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. *Circulation.* 2000;102(19):2347-2352.
307. Kim MH, Bu SY, Choi MK. Daily calcium intake and its relation to blood pressure, blood lipids, and oxidative stress biomarkers in hypertensive and normotensive subjects. *Nutr Res Pract.* 2012;6(5):421-428.
308. Grundy SM. Low-density lipoprotein, non-high-density lipoprotein, and apolipoprotein B as targets of lipid-lowering therapy. *Circulation.* 2002;106(20):2526-2529.
309. Langsetmo L, Berger C, Kreiger N, 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.